ANTIMICROBIAL PHARMACOKINETICS IN ENDOPHTHALMITIS TREATMENT: STUDIES OF CEFTAZIDIME*

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INTRODUCTION

THE RESULTS OF TREATMENT FOR ENDOPHTHALMITIS WERE USUALLY DISappointing before the introduction of vitrectomy and the widespread acceptance of intraocular antibiotic administration. Leopold[1] reviewed 103 cases reported in the literature from 1944 to 1966 and reported that 73% had a posttreatment vision of hand motions or less. During recent years, significantly better results have been achieved. Improvement is a consequence of both improved therapeutic approaches and a trend toward less virulent infections.[2-6]

Injection of antibiotics into the vitreous cavity (which began with sulfa compounds and penicillin)[7-12] finally became fully accepted 35 years later and is now considered indispensable in the treatment of endophthalmitis.[2-5,13] Vitrectomy is commonly an important part of the therapeutic intervention, but its role remains more controversial.[14]

Many issues remain in the antimicrobial therapy of endophthalmitis. Definition of standards for toxicity after intraocular antimicrobial injection, the efficacy of single intravitreal injections for complete cure of endophthalmitis, and the role of multiple injections of antimicrobials require further clinical and laboratory study. Antibiotics are frequently given intravenously either prophylactically or as part of the treatment regimen, but their intraocular penetration and contribution to therapeutic success remain uncertain.[14,15]

Studies of pharmacokinetics of antimicrobials for the treatment of endophthalmitis are fundamental to arriving at recommendations for clinical therapy. Typically, penetration of antimicrobials into the eye after intravenous administration (Table I) or clearance of an antimicrobial from the eye after intravitreal injection (Table II) is studied after a single bolus of drug is given, followed by sampling of the concentrations in a phakic

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TABLE I: ANTIBIOTIC CLEARANCE AFTER INTRAVITREAL INJECTION (μg/ml)

DRUG	MODEL	DOSE	PHAKIC			APHAKIC			APHAKIC-VITRECTOMIZED			REFERENCE
			24 HR	48 HR	T½ HR	24 HR	48 HR	T½ HR	24 HR	48 HR	T½ HR	
Carbenicillin disodium	Monkey, normal	1 mg	50*	11*	10†							41
Cefazolin sodium	Monkey, normal	1 mg	350*	30*	7‡							41
	Rabbit, infected	2.25 mg	8.7	3.15								107
	Rabbit, control	2.25 mg	147	8.97	6.5	183.3	17.7	8.3	25.7	3.7	6	19
	Rabbit, inflamed	2.25 mg	340	57.4	10.4	242.5	31.9	9.0	33.4	5.7	6.7	19
Cefriaxone sodium	Rabbit, normal	2 mg	200	45								72
	Monkey, normal	2 mg	434	59								108

Vancomycin	Rabbit	2 mg	> 100	> 100					90§	16§		40
Amikacin	Rabbit, normal	250 µg	19	7								109
	Rabbit, control	400 µg	100.9	55.6	25.5	25.3	15.3	14.3	15.5	3.0	7.9	18
	Rabbit, inflamed	400 µg	97.6	31.4	15.5	7.6	1.5	7.4	7.2	1.4	7.7	18
Gentamicin	Human, infected	100 µg					4.6					39
	Monkey, normal	100 µg	10.5	6.5	34							41
	Cat, normal	400 µg	50									110
	Cat, infected	400 µg	11									110
	Rabbit, control	71 µg/ml°	75	40	32	25	7	12				39
	Rabbit, infected	40-65 µg/ml°	35	18	19	20	6	14				39

°Estimated from graphic representation.
†With probenecid T½ = 20 hours.
‡With probenecid T½ = 30 hours.
§Without intact capsule.
Modified from Gardner.[111]

TABLE II: VITREOUS VOLUME AND AQUEOUS FLOW

SPECIES	VITREOUS VOLUME (ml)	AQUEOUS FLOW (μl/min)
Rabbit	1.4-1.7	3.6
Cat	2.4	13.0
Dog	3.2	
Monkey	3.0-4.0	3.0
Man	3.9-5.0	2.5-3.0

Modified from Barza.[27]

noninflamed eye. The pharmacokinetics of antimicrobials in the vitreous cavity are changed significantly by prior ocular surgery, by inflammation, and by multiple intravenous dosing regimens.[16-19] Thus the results in phakic noninflamed eyes may have little relevance for the usual clinical situation. To more fully understand the kinetics of intraocular microbials, studies taking these variables into account are mandatory.

Prophylaxis and therapy of gram-negative endophthalmitis remains an important problem. In this thesis, results of studies on the intraocular pharmacokinetics of ceftazidime, a third-generation cephalosporin, in normal and surgically altered eyes are reported. The study design, which includes both control and inflamed eyes, analyzes the effects of a multiple intravenous dosing schedule on intraocular drug concentrations.

BACKGROUND AND SIGNIFICANCE

The goal of antimicrobial therapy for endophthalmitis is to provide adequate intravitreal concentrations of appropriate antimicrobials for a sufficient time to eradicate micro-organisms while avoiding concentrations of drug that can produce iatrogenic tissue damage. In the very recent past, however, the choice of antimicrobial and the route of administration were determined only by the spectrum of antimicrobial coverage and by rudimentary toxicity considerations. Antimicrobials have often been treated in ophthalmology as if they are antiseptics, which by definition kill on contact, rather than requiring a specific concentration in tissue or fluid for a certain period. Although intravitreal antibiotics are now considered standard therapy for endophthalmitis, little attention has been paid to the prolonged intravitreal availability of antimicrobial agents. Despite lack of evidence for a contribution to therapeutic concentrations within the vitreous cavity, prophylactic and therapeutic administration of intravenous antibiotics has become routine and is sometimes considered the standard of care in many clinical situations.

A main objective of therapy of endophthalmitis is rapid sterilization of the vitreous cavity. Factors in antimicrobial selection for endophthalmitis ther-

apy should include not only spectrum of coverage and intraocular toxicity but also duration of effective dose within the eye after intravitreal injection, intraocular penetration after intravenous administration, and in vivo antimicrobial activity. Underlying the controversy about the effectiveness of single-injection antibiotic therapy in endophthalmitis is lack of knowledge of the concentration and duration of exposure necessary in the vitreous cavity for various antimicrobials to achieve rapid bacterial killing. This subject has received scant research attention, but analogies to the treatment of meningitis are probably pertinent, since infections in the cerebrospinal fluid (CSF) and vitreous cavity share a number of characteristics.

ENDOPHTHALMITIS AND MENINGITIS

Meningitis and endophthalmitis are analogous in a number of their features. In both instances, infection takes place in a closed space, carrying significant implications for treatment and outcome. Bacterial replication and inflammation may cause tissue destruction quickly in both cases, leading to significant functional loss in spite of successful eradication of the invading organism.[20-22] In both conditions, it is necessary to choose antibiotics and start therapy empirically prior to identification of the organism to attempt to control the infection as quickly as possible.[21] Penetration of antimicrobials into the CSF and into the vitreous cavity after intravenous administration is limited by the blood-brain barrier[20,22-24] and by blood-ocular barriers,[25-27] respectively. Certain antimicrobials are ineffective because of inadequate penetration. Furthermore, bacterial killing in the CSF may require higher concentrations of antimicrobials,[10,28,29] a requirement not yet studied in the vitreous cavity. Higher concentrations may be necessary in part due to the immune privilege of the CSF, a condition also found in the eye.[20] A relative immune privilege has been demonstrated for the anterior chamber, known as anterior chamber autoimmune deviation (ACAID), and has been suggested for the posterior segment as well.[30,31] Immune privilege with regard to infections within the vitreous cavity has not yet been well studied or characterized.

THERAPEUTIC CONCENTRATION RANGE

The therapeutic concentration range or therapeutic window is the range of drug concentration associated with effective therapy without undue toxicity.[32] For most drugs, the therapeutic concentration range in plasma is narrow and the upper and lower limits differ by a factor only 2 or 3. The upper limit of the concentration may be a result of diminishing effectiveness of the drug at higher concentrations or may be established by toxicity. Toxicity, in turn, may be an extension of the pharmacologic properties of the

drug or may be totally disassociated from its therapeutic effect. The narrower the range within which the chances of successful therapy are high, the more difficult the maintenance of values within this range. The issue of toxicity as the limiting factor in the upper range of drug concentrations is particularly meaningful with intravitreal injections, since very high initial concentrations are achieved, and in other tissues toxicity is often related to peak dose.

BASIC PHARMACOKINETICS

The intravitreal concentration of antibiotic at a given time is governed by several factors.[32] Following an intravitreal injection of a drug, the initial concentration is a result of the dose and the extent of distribution. Afterward the concentration of the drug at a given time is determined by the volume of distribution, the dose of the initial injection, and the rate of elimination. The elimination phase of the drug may be characterized by two parameters: (1) the apparent volume of distribution and (2) the elimination half-life.

The volume of distribution is a direct measure of the extent of distribution of the drug but rarely corresponds to a real volume. The volume of distribution may be many times higher than the actual physical volume or may be lower, depending on a number of factors. The half-life (T½) is defined as a period of time required for the drug concentration to fall by one half. Elimination of a drug from the body, a tissue, or a compartment is usually a first-order process, and by definition the rate of the elimination is proportional, therefore, to the amount of drug present. When plotted on semilog paper, the elimination of drug is usually a linear function. The elimination constant, k, is a definition of the fractional rate of drug removal. K is a first-order rate constant with a dimension of time (-1). K may be defined as the rate of elimination divided by the amount of drug in the body. The half-life then may be expressed as $T½ = 0.693/k$.

Clearance is the parameter that relates the concentration to the rate of drug elimination. The rate of elimination equals clearance multiplied by concentration. The units of clearance are given in volume per unit of time and the half-life may be expressed as:

$$T½ = 0.693 \times \text{volume of distribution} \div \text{clearance}$$

Both the half-life and elimination rate constant reflect, rather than control, the volume of distribution and the clearance of the drug.

Implicit in the concept of the half-life as a first-order process is that less drug is eliminated with each succeeding half-life. On a practical level, all

drug (97%) can be regarded as being eliminated within five half-lives. In systemic administration, the interval chosen for dosing is approximately every four half-lives, since the concentration will usually fall below effective levels before the time of the fifth half-life is reached. After intravenous injection of antimicrobials, the rate of distribution of the drug between blood and tissue is limited by either perfusion or permeability, depending on the organ being analyzed. The choroid has the highest blood flow rate in the body,[33,34] but there are significant barriers to movement of drugs into the retina and vitreous. The entry of drug into the vitreous cavity, therefore, is predominantly a permeability-limited function. If the concentration of the drug in blood is maintained long enough, any drug should reach a distribution equilibrium where the concentration in tissue and plasma are equal. However, equality is not always observed, for reasons such as active transport out of the tissue (a factor with beta-lactam antibiotics in the eye)[16,25,35] and pH gradients across cell membranes. When there is a decreased rate of entry into the tissue (eg, because of the blood-brain barrier), the time to reach a distribution equilibrium is increased; the point of this equilibrium, however, is independent. The approach to the plateau concentration within a cavity such as the eye is determined only by the tissue distribution half-life and may be expressed by the following formula:

$$\text{half-life} + 0.693\ k_t = 0.693\ k_p \div Q/v_t$$

where k_t = fractional rate of exit with units of reciprocal time, k_p = equilibrium distribution ratio, Q = blood flow, and v_t = volume of tissue.

Within the eye, the concentration of drug at any given time is a combination of the amount given by intravitreal injection plus the inflow of drug through aqueous or through posterior structures, reduced by the outflow through the trabecular meshwork, across retinal structures by passive diffusion, and across retinal structures by active transport.

PHARMACOKINETICS OF INTRAVITREAL INJECTIONS

Once antibiotics are injected into the eye, they diffuse through the vitreous cavity without significant barriers and are eliminated from the eye by either an anterior or a posterior route.[25,26,36] Drugs exiting from the anterior route are removed by the flow of the aqueous humor or by diffusion across the iris surface. Drugs must move through the vitreous cavity, around the lens when it is present, and into the anterior chamber, and then exit through the trabecular meshwork into the canal of Schlemm (Fig 1). Aminoglycosides, streptomycin sulfate , vancomycin, and sulfacetamide sodium are thought to be eliminated anteriorly.[25,26] The posterior route or "retinal route" is the

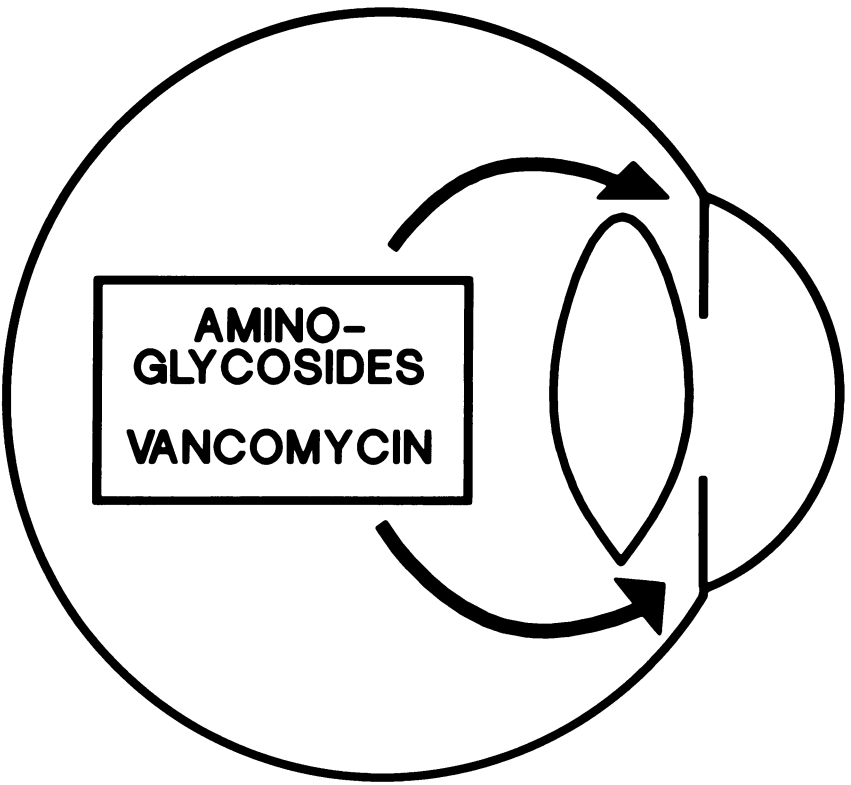

FIGURE 1
Route by which antimicrobials, including aminoglycosides and vancomycin, leave eye through anterior chamber following intravitreal injection.

alternative pathway for drug elimination from the vitreous cavity (Fig 2). The first- and second-generation cephalosporins, clindamycin and dexamethasone, are believed to be eliminated posteriorly.[25] Posteriorly, there is a barrier between the vitreous humor and the retina thought to be shared between the retinal capillaries and the pigment epithelium.[25,26,36,37] This barrier is normally impermeable to materials of high molecular weight, but there is active transport of numerous substances out of the vitreous by the retinal structures. Both anterior and posterior routes of egress may come into play for some substances; ceftriaxone sodium has been suggested as one example.

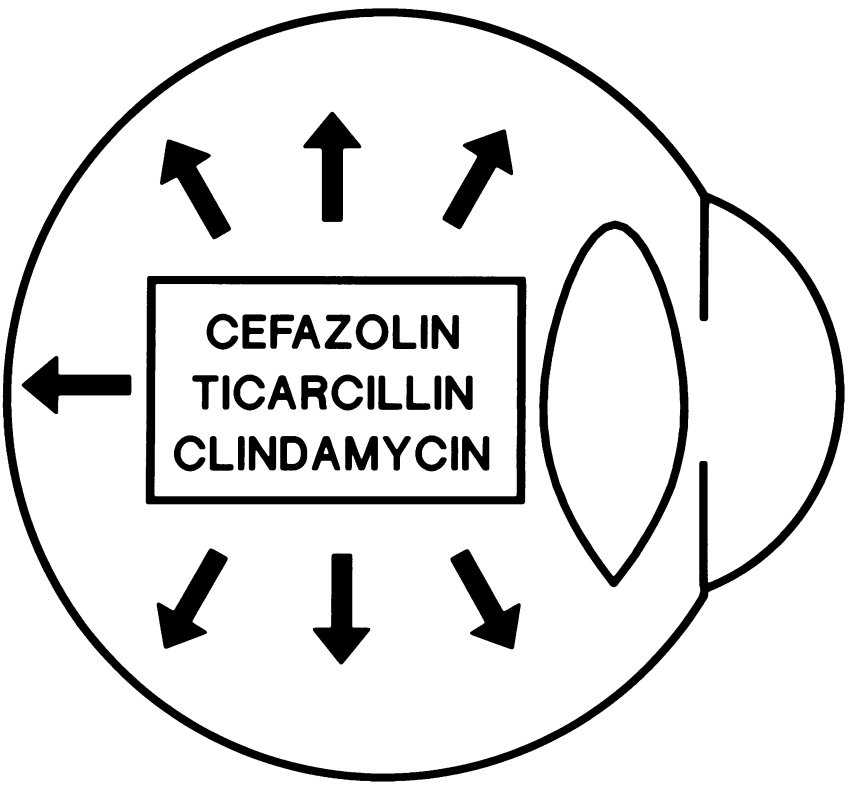

FIGURE 2
Posterior route of elimination of drugs, including clindamycin and many cephalosporins, from vitreous cavity.

According to Maurice, antibiotics diffuse through the vitreous rapidly after intravitreal injection, although this may take several hours.[26,36] Lack of resistance to diffusion of drugs within the vitreous cavity is due to low average concentration (0.01%) of collagen in the vitreous gel, and fluid flow is thought to be less important than movement by molecular action. Maurice states that if elimination is entirely through the anterior chamber, the amount of drug lost from the vitreous body in 1 hour equals:

$$(k_v) \times (c_v) \times (v_v) = f \cdot c_a$$

where k_v is the fraction lost every hour, c_v is the average concentration of the vitreous body, and v_v is the volume in the vitreous body, f is the volume of the aqueous flow in 1 hour, and c_a is the concentration of drug in the aqueous. The rate of loss in this situation is thought to be almost entirely controlled by the rate of diffusion within the vitreous body, which is determined predominantly by geometric factors. Maurice has calculated that injecting 100 times the therapeutic concentration of a drug into the central vitreous cavity allows it to reach the therapeutic concentration at the retinal surface within about 3 hours.

On the other hand, recent studies of drug elimination from rabbit eyes demonstrate that removal of the vitreous significantly shortens the half-life of several types of antimicrobials, suggesting the importance of the vitreous for retaining antibiotic after it has been injected. In studies of amikacin, Meredith and associates[18] demonstrated that the half-life was reduced from 14.3 hours after injection of 400 μg into the aphakic eye to 7.9 hours after removal of the lens and vitreous. Martin and associates[16] demonstrated a reduction in half-life from 8.3 to 6.0 hours when comparing clearance of intravitreal cefazolin from phakic versus aphakic-vitrectomized eyes (Table I). Doft and associates[38] noted that the half-life of intravitreal amphotericin was reduced from 4.7 days in aphakic eyes to 1.4 days in eyes with both lens and vitreous removed.

Maurice suggested that as a drug leaves the vitreous by the anterior route, the lens creates a bottleneck, slowing removal from the eye. For this reason, half-lives of anteriorly excreted drugs are longer than those of posteriorly excreted drugs. Subsequent experiments have shown that removal of the lens in rabbits decreases the half-life of intravitreal amikacin from 25.5 to 14.3 hours[18] and of intravitreal gentamicin from 32 to 12 hours[39] (Table I). Vancomycin has a shorter half-life in aphakic than in phakic eyes; the half-life is intermediate between these two values when the lens is removed but the posterior capsule left intact.[40]

Inflammation also decreases the half-life of anteriorly excreted drugs, although the mechanism that accounts for this is not clear. In phakic rabbit eyes, the half-life of amikacin is decreased from 25.5 to 15.5 hours by inducing inflammation,[18] while the half-life of gentamicin is decreased from 32 to 19 hours.[39] The same effect is noted in aphakic rabbit eyes for amikacin; the half-life was reduced from 14.3 to 7.4 hours by induction of inflammation.[18] No prolongation of half-life was noted for gentamicin in aphakic rabbit eyes by inducing infection.[39] Inflammation may increase posterior permeability, and these drugs may then be eliminated by both anterior and posterior routes, accounting for the observed increase in rate of elimination.

Substances removed posteriorly are thought to exit through the retinal vasculature and/or retinal pigment epithelium (RPE). For these drugs, Maurice[26,36] suggests that the diffusional path through the vitreous is shortened and the anterior bottleneck provided by the lens is now replaced by a wide surface area available for absorption. Because active transport may be involved in excretion of some substances posteriorly and there is a wide surface area for absorption, posteriorly excreted drugs usually have a significantly shorter half-life than do anteriorly excreted ones.[25] The system may demonstrate the properties of saturation kinetics; competitive inhibition and metabolic inhibition have been demonstrated in the case of beta-lactam antibiotics. It has been suggested that there is some correspondence between the extent of renal tubular excretion of drugs in humans and the vitreous half-life in rabbits.[26,35] Probenicid administration, for example, prolongs the half-life of intravitreal carbenicillin from 5 to 13 hours in the rabbit and from 10 to 20 hours in the monkey.[41] The half-life of cefazolin is prolonged from 7 to 30 hours in the monkey by probenecid.[41]

Inflammation has also been noted to increase the half-life of cefazolin injected intravitreally, probably by interfering with active transport. In phakic rabbit eyes the half-life was increased from 6.5 to 10.4 hours by inflammation.[19] As the posterior route is blocked by metabolic or competitive inhibition, the anterior route of removal may become more important or even become the major route of elimination from the eye. The half-life for cefazolin in the monkey, for example, in the presence of probenecid is 30 hours,[41] a value essentially the same as the half-life of gentamicin.[39] Inflammation may have more complex effects, however, since the permeability of the posterior structures may be increased, offsetting the effect of disabling active transport. In the case of cefazolin, aphakic and aphakic-vitrectomized eyes have similar half-lives when comparing control to inflammation.[19] The effect of vitreous removal appears to predominate over inflammation for cefazolin and for amikacin in aphakic and aphakic-vitrectomized eyes. The values for half-life are 6 to 8 hours for both inflamed and control eyes for these two antibiotics.[18,19]

No information is available on elimination times of antimicrobials after direct injection in the human eye. Some data are derived from studies in monkeys, but the majority of information is available from studies done in the rabbit eye (Table I). Typically in these studies, the animal eyes are injected with the dose used in the human eye. The volume of the vitreous cavity has considerable intraspecies differences (Table II). Since the volume of the human eye is approximately 4 cc and that of the rabbit is 1.4 cc, the initial concentration of the study drug is then approximately 2.8 times higher in the rabbit than would be expected in the human. Maurice has

postulated that since the vitreous cavity volume is significantly greater in the human than in the rabbit, the times of diffusion to the retinal surface are expected to be greater. He estimates that the half-life of a drug in a human may be 1.7 times longer than that in the rabbit vitreous cavity.[26,36] Recommended initial dosage of antimicrobials for intravitreal injection range from 100 μg for gentamicin to 2.25 mg for cefazolin. Assuming an average vitreous volume of 4 cc, initial concentrations, therefore, vary from 25 μg/ml for gentamicin to 562 μg/ml for cefazolin.

As a theoretical example, 100 μg of a drug injected into the rabbit vitreous with a volume of 1.4 cc creates an initial concentration of 71 μg/ml. If the half-life of drug is 20 hours, the concentration of drug in the eye after 100 hours (five half-lives) is approximately 2.1 μg. The same amount of drug injected into the human eye with a volume of 4 cc yields an initial concentration of 25 μg/ml. If the half-life is 1.7 times longer for the human than the rabbit, it will be 34 hours. After 100 hours (three half-lives) the concentration will be approximately 3.1 μg/ml. Therefore, data obtained from the rabbit approximate the values to be expected in the human for the time for drug to fall to low concentrations although other factors, such as differences in protein-binding between the rabbit and human, may cause further discrepancies.

BLOOD-OCULAR BARRIERS

Penetration of drug into the eye from plasma is restricted by several barriers. The permeability restriction between blood and aqueous that excludes certain substances either actively or passively is termed the blood-aqueous barrier.[26,37] The blood-aqueous barrier is thought to be similar to the blood-brain barrier, since there is apparent active inward transport of some substances in addition to the exclusionary properties. In the posterior pole there are two major barriers to penetration, the retinal capillary endothelial cells (also called the inner blood-retinal barrier) and the tight junctions of the RPE (sometimes termed the outer blood-retinal barrier).[25,26] The epithelium of the ciliary body where it faces the vitreous may also serve a barrier function.[37]

The blood-brain barrier has been extensively studied, but less information is available on the blood-retinal barriers, although they are thought to have similar properties because of similar anatomic features.[20,25,26] In the retinal capillaries the tight junctions between endothelial cells create the blood-retinal barrier. Most other capillaries in the body, except in the central nervous system and prostate, are fenestrated and have pores large enough to admit substances of molecular weight up to 1000 daltons (ds). Retinal capillaries have tight junctions and are nonfenestrated, creating an obstruc-

tion to movement of substances from plasma into the retina and vitreous. The outer blood-retinal barrier is produced by the tight junctions between the cells in the RPE. There is a high rate of blood flow to the choriocapillaris, and its surface area is estimated to be about 2.5 times the surface of the choroid.[26] These capillaries are extremely permeable, but substances leaking out encounter the barrier of the junctional complexes between retinal pigment cells and the pigment epithelium cells in the pars plana.

When drugs must pass through epithelial cells, they must cross cell membranes, which create selective barriers to penetration by various substances. Most cellular membranes have a central layer that is predominantly lipoidal in nature, bounded on each side by protein layers.[20,32] Drug penetrations throughout the body are a result of passive diffusion across barriers of this kind. The rate of penetration equals the permeability times the surface area times the concentration gradient.[26] Higher lipid solubility allows antimicrobials to pass more easily through lipid membranes of capillary epithelium. Lipid-soluble drugs include minocycline, doxycycline, chloramphenicol, trimethoprim, and metronidazole (Appendix I). Drugs that are less lipid-soluble include those that are more commonly given locally and systemically for endophthalmitis, the beta-lactams and the aminoglycosides.[20,25]

The pH of the system also influences the barrier effect, since ionized molecules are more polar than nonionized molecules, and therefore are less soluble in the lipid membrane.[22,32] Molecules that are more ionized are, therefore, less able to travel across the blood-retinal barrier.[32] There is an increased accumulation of drug on the side of the membrane whose pH favors greater ionization. The pH partition hypothesis states that only nonionized, nonpolar drugs penetrate membranes and that the concentration of nonionized species is equal on both sides of the membrane.[32] This affects the penetration of cephalosporins, for example, which are more ionized than many drugs at physiologic pH.[42]

Molecular weight affects penetration of drugs across barriers. The larger the molecule and the higher the molecular weight, the less readily can the molecule cross the blood-retinal barrier. The ability to pass through membranes is related to the square root of the molecular weight, and most drugs fall in a narrow range of molecular weight between 100 and 400 ds. In general, this is a less important effect in determining crossing of barriers.[20,22,26]

Protein-binding can also restrict passage of molecules out of a vascular compartment, since it is generally held that only unbound antimicrobials can pass through membranes.[20,22,26] Many antimicrobial agents are extensively bound to serum proteins and particularly to albumin. Protein-binding

APPENDIX I: CHARACTERISTICS OF SELECTED ANTIBIOTICS

DRUG	LIPID SOLUBILITY	ESTIMATED % INTRAOCULAR PENETRATION*		ROUTE OF INTRAOCULAR ELIMINATION	MECHANISM OF ACTION: INHIBITS	BACTERIAL KILLING CHARACTERISTICS
		UNINFLAMED	INFLAMED			
Aminoglycosides	Weak	3-9	4	Anterior	Protein synthesis	Concentration-dependent
Chloramphenicol	Good	16			Protein synthesis	Bacteriostatic
Clindamycin	Weak	10		Posterior	Protein synthesis	
Cephalosporins (first & second generation)	Weak			Posterior	Cell wall	Time-dependent
Cephalosporin (third generation)	Weak			Anterior & posterior	Cell wall	Time-dependent
Minocycline	Good				Protein synthesis	Bacteriostatic
Quinolones	? Weak	4			DNA synthesis	
Vancomycin	Poor			Anterior	Cell wall	Concentration-dependent

*Approximate peak intraocular concentration as a percentage of simultaneous serum concentrations.
Data from Barza,[25] Maurice and Mishima,[26] Spivey,[76] Vogelman and Craig,[77] and Zhanel and associates.[78]

is reversible and rapid, reaching an equilibrium quickly. In the case of cephalosporins, there is variable protein-binding of the third-generation cephalosporins and the degree of protein-binding correlates inversely to the percentage of penetration into CSF.[43]

Active transport mechanisms achieve net movement of drug against a concentration gradient and, as previously mentioned, are involved in transporting some microbials both out of and into the CSF; transport may thus be bidirectional. There is evidence of active transport of ceftriaxone, an important cephalosporin, into the CSF, but transport into the eye has not been demonstrated in a similar fashion.

Inflammation may play a major role in the breakdown of the blood-brain barrier by disrupting tight junctions and increasing transendothelial vesicles, increasing pinocytosis, and enhancing the formation of microvilli by endothelial cell luminal membranes.[20] Many drugs penetrate into the CSF more easily when the meninges are inflamed, and inflammation has also been shown to increase the penetration of antimicrobials into the eye. For cefazolin, for example, no drug penetrates into the phakic noninflamed eye even after repeated doses, but levels gradually increase to an average of 10.9 μg/ml in the inflamed eye after seven intravenous doses on an every-8-hour administration schedule.[16] This effect on permeability may be important early in the course of infection, but as inflammation decreases during the course of the disease, permeability and thus antibiotic penetration may also decrease.[25]

Other factors have also been shown to affect penetration into the vitreous cavity. By removing the lens and vitreous, the inner eye is converted to a single chamber. In this configuration drug can enter the vitreous cavity both through the aqueous and through posterior structures. After removal of the vitreous and lens, the concentration of cefazolin was found to be higher in the vitreous cavity after intravenous administration than in eyes without the vitreous removed, presumably owing to the removal of a physical barrier to diffusion added to a contribution from aqueous humor (phakic = 0 μg, aphakic-vitrectomized = 3.9 μg/ml).[16] When coupled with inflammation, this effect was more striking, and in the inflamed eye with vitreous removed, there is a significantly higher concentration achieved in the vitreous cavity than there is in eyes with intact vitreous (phakic inflamed, 10.6 μg/ml versus aphakic-vitrectomized, 24.9 μg/ml)[16] (Fig 3).

When repeated intravenous doses are given, intravitreal drug concentrations may progressively increase with time, because the barrier to diffusion increases the time needed to reach a point of equilibrium.[32] This is a potentially important effect, because in most studies of drug penetration, a single bolus dose of antimicrobial is given intravenously followed by subse-

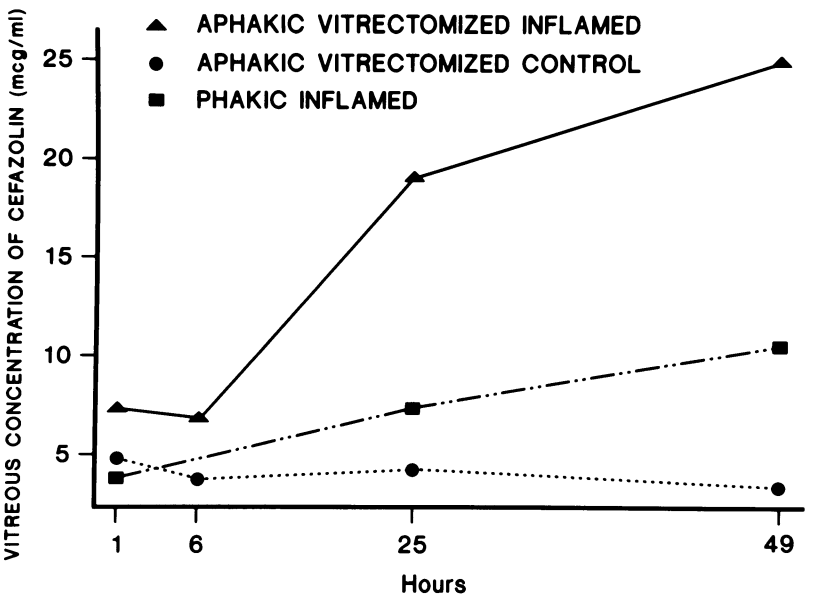

FIGURE 3
Vitreous concentrations of cefazolin during intravenous administration every 8 hours.

quent sampling 1 to 2 hours later. Studies on gentamicin and on cefazolin demonstrated progressively higher concentrations over time with repeated intravenous drug administration.[16,44]

The penetration of an antibiotic into the eye or the CSF is usually expressed in terms of the percentage of the drug at a given time compared with the amount of concentration of the drug in serum (CSF ÷ plasma × 100).[24] Problems exist in this determination, however, since drug concentrations may change slowly in the vitreous and other body cavities but more rapidly in the plasma. The precise time of sampling of drugs is, therefore, significant in establishing a percentage.

Aminoglycosides,[45,46] beta-lactam agents,[46] and vancomycin[40,47-49] are probably the antimicrobials most commonly administered intravenously for endophthalmitis. No data are available for the penetration of vancomycin into the eye. A number of studies of aminoglycosides indicate little, if any, penetration under various conditions. Studies of vitreous sampling in humans after single intravenous doses do not demonstrate therapeutic levels of gentamicin in the vitreous cavity.[50] Barza and associates[44] studied phakic infected rabbit eyes and were able to demonstrate concentrations of 2.6 μg/ml after continuous intravenous infusion of gentamicin for 6 hours,

reaching slightly higher levels and a penetration ratio of 42% after 18 hours. Yoshizumi and associates[51] studied a model of traumatized eyes in the rabbit and could not demonstrate a therapeutic level of gentamicin in the vitreous cavity after intravenous injection. In our laboratories intermittent intravenous doses of amikacin produced vitreous cavity levels of approximately 2 μg/ml in aphakic-vitrectomized inflamed rabbit eyes; these levels are less than the minimum inhibitory concentration (MIC) for target organisms (unpublished data).

Studies have been done of human samples obtained by vitrectomy at various intervals after a single intravenous bolus dose of cefazolin sodium, methicillin sodium, cephalothin sodium, oxacillin, and nafcillin sodium.[52] Only cefazolin produced vitreous concentration consistently above the MIC for *Staphylococcus epidermidis*, but did not reach effective levels for *Staphylococcus aureus*. Similar studies demonstrated inadequate penetration of moxalactam disodium to achieve levels above the MIC for *S aureus* or *S epidermidis*; cefamandole nafate did not reach therapeutic concentrations for gram-negative pathogens consistently.[53] Imipenem tested under similar circumstances exceeded the MIC for *S aureus* and *S epidermidis* for some but not all patients, but did not reach the MIC levels for methicillin-resistant *S aureus* or for many important gram-negative pathogens causing endophthalmitis.[54] In studies of human vitreous samples after oral administration of ciprofloxacin in patients undergoing vitrectomy for various indications, the average vitreous concentrations did not exceed the MIC for *S aureus, Streptococcus pyogenes* or *Pseudomonas aeruginosa* consistently[55,56] (Table III). Most eyes on which human studies have been performed did not have infections, but many had conditions such as trauma and proliferative diabetic retinopathy, which might be expected to break down the blood-ocular barriers. Similar low values are found in animal studies, although inflammation increases the degree of penetration.

PHARMACOKINETICS OF SUBCONJUNCTIVAL INJECTIONS

Because the RPE is a barrier to diffusion, after subconjunctival injection antimicrobials enter the vitreous cavity poorly.[25] There is evidence for higher levels of antimicrobials in the aqueous after subconjunctival injection, but insufficient levels are produced in the vitreous cavity to significantly enhance antimicrobial effects for most antibiotics.[57-62] In studies of subconjunctival injections of third-generation cephalosporins, the corneal levels achieved were fourfold higher than aqueous concentrations. Concentrations in the choroid were fivefold to 15-fold higher than for the retina; retinal concentrations were approximately 10-fold higher than vitreous cavity. Thus, there is a significant concentration gradient from choroid to retina

TABLE III: INTRAOCULAR PENETRATION OF ANTIBIOTICS AFTER SYSTEMIC ADMINISTRATION (μg/ml)

DRUG	MODEL	DOSE	PHAKIC			APHAKIC			APHAKIC-VITRECTOMIZED			REFERENCE
			1-3 HR	24-25 HR	49 HR	1-3 HR	24 HR	49 HR	1-3 HR	25 HR	49 HR	
Cefazolin sodium	Rabbit, control	50 μg/kg q8h × 48 hr	0	0	0				4.2	3.7	2.1	16
	Rabbit, inflamed	50 μg/kg q8h × 48 hr	3.0	6.3	10.6				6.7	19.0	24.9	16
	Human, vitrectomy	2 g IV	0.84-1.6									52
Ceftriaxone sodium	Human, vitrectomy	1-2 g IM	5.9	11.5								52
Ceftazidime	Rabbit, control	50 mg/kg × 1	0.5-0.1									101
	Rabbit, inflamed	50 mg/kg × 1	3.1-1.2									101
Amikacin	Rabbit, control	15 mg/kg				0.9						112
	Rabbit, inflamed	15 mg/kg				Variable						112
Gentamicin	Human, vitrectomy	1.5 mg/kg IM	< 0.2									50
	Rabbit, trauma	2.29 mg	0.25									51
	Rabbit, infected	4.6 mg/kg IM q3h × 5		2.8								44
Ciprofloxacin	Human, vitrectomy	750 mg PO		0.04-0.49								55
Imipenem	Human, vitrectomy	1 g IV	2.53									54

and from retina to vitreous. When the eyes were inflamed in this study, the barriers appeared to be preserved. This report suggested that subconjunctival injections cannot replace the role of intravitreal injections in the treatment of endophthalmitis.[35]

TOXICITY OF INTRAVITREAL ANTIBIOTICS

Tissue toxicity is one determinant of the upper end of the therapeutic range.[32] Because intravitreal injections often result in intraocular concentrations much higher than routinely achieved elsewhere in the body by intravenous dosing, toxicity considerations are particularly important in the eye.

Concentrations of gentamicin within the CSF after single intrathecal injection are reported to be between 27 and 81 μg/ml. Estimated intravitreal concentrations are 100 μg/ml when 400 μg of gentamicin is injected into the vitreous cavity or 25 μg/ml when 100 μg is injected. In one study of neonatal gram-negative bacillary meningitis, a prospective study demonstrated that infants treated with intrathecal injection and systemic medications had a higher mortality rate than those treated with systemic medication alone. One possible explanation for higher mortality rate with intrathecal therapy was either iatrogenic damage or gentamicin toxicity due to higher concentrations after intrathecal injection.[23]

Within the eye, criteria for toxicity have not been completely defined in the posterior segment. Histopathologic criteria are most frequently employed and involve demonstration of changes in the retina or RPE at some time after injection of intravitreal antimicrobials. The rabbit has been chosen for most toxicity studies, usually for reasons of expense and convenience. However, it has been demonstrated that this may be an inadequate model, since the rabbit retina is merangiotic with less vasculature, as compared with the holangiotic retina of a higher-level primate. In testing for toxicity of aminoglycosides, this difference in structure led to failure to recognize the vascular obliterative complications, which are now thought to be not only the most common sign of toxicity but the most damaging problem after intraocular aminoglycoside administration.[46,63]

The electroretinogram may also be used as a criterion for toxicity, but its efficacy is complicated by the fact that surgical invasion of the eye can lower the electroretinographic response. Therefore, it is very important to include careful concurrent controls in which placebo is injected simultaneously.[64]

Toxicity may be due to the antimicrobial itself or to the vehicle or to the preservatives associated with it. Marmor[65] has also suggested that changes of pH or osmolality may create iatrogenic tissue damage to the retina. This risk is probably minimized by the low volumes of drug injected, which are typically only 0.1 ml.

Peyman and associates[66] have hypothesized that injection of antibiotic into the eye in which the vitreous has been removed may increase the risk for toxicity. It is postulated that toxicity may occur because the antibiotic settles on the retinal surface, causing a high dose at the retinal surface rather than mixing completely within the vitreous cavity. Comparison of rates of toxicity in vitrectomized versus nonvitrectomized eyes has been made in studies of aminoglycosides,[67] and no difference has been demonstrated in toxicity on histopathologic criteria.

The antibiotics that have been studied most carefully for their toxic potential after intraocular injection are the aminoglycosides. Gentamicin has been most completely characterized. Zachary and Forster[68] noted dose-related damage to the outer retina with marked disruption of the outer nuclear layer and prominent loss of outer segment with doses exceeding 0.2 mg injections in the rabbit. Ophthalmoscopically, retinal pigment epithelial mottling, clumping, and scattered areas of depigmentation were noted. The electroretinogram (ERG) became extinguished when doses of 0.4 to 0.5 mg were given. Talamo and colleagues[45] studied electron microscopy specimens from the rabbit after gentamicin intravitreal injections and noted lamellar lysosomal inclusions similar to drug-induced lipid storage problems. These changes were similar to findings in the kidney and were thought to suggest that RPE is the primary site of toxicity for gentamicin.

Initially no vascular abnormalities were noted on animal testing until Conway and Campochiaro[63] identified a syndrome of infarction of macular vessels in humans due to intraocular injection of gentamicin. A subsequent survey of retinal specialists identified a number of similar cases secondary to both gentamicin and amikacin.[46] To further characterize this complication in the primate, Conway injected 1000 μg of gentamicin in *Cebus navrigatus* monkeys.[69] Three days later the clinical picture of macular infarction was noted with cotton-wool spots and intraretinal hemorrhages. On electron microscopy there was striking damage to the inner retinal layers, mainly the nerve fiber, ganglion cell, inner plexiform, and nuclear layers. There were less severe changes in the outer layers. Despite the picture of macular infarction clinically, there were no apparent vascular changes. The investigators suggested that neurotoxic changes lead to shutdown of regional blood flow, perhaps through granulocytic plugging in these cases.

D'Amico and associates[70] studied five aminoglycosides after intravitreal injection, examining the clinical picture, histopathology, and electron microscopy. They noted that the first abnormality produced was lysosomal overloading of the RPE with lamellar lipid material. At doses of 1500 μg of amikacin and 400 μg of gentamicin, toxic reactions in the outer retina were noted: macrophages in the subretinal space with storage lysosomes, disorga-

nization of the photoreceptor outer segments with preservation of the inner segments, and focal necrosis of the RPE. There were areas of focal disappearance of photoreceptors and RPE with reactive gliosis as a late finding. Doubling these dosages led to full-thickness retinal necrosis marked ophthalmoscopically by grey-white areas in the RPE corresponding to destruction of the photoreceptor-RPE complex. In this model the relative toxicities established were gentamicin > netilmicin sulfate = tobramycin > amikacin = kanamycin sulfate.

Repeated doses of intraocular antibiotics may increase the risk for complications. In other systems in the body the antibiotic toxicity is often related to peak dose. With multiple intraocular injections, high peak doses are repeated. Oum and associates[71] injected 1 mg of vancomycin along with amikacin (400 μg) or gentamicin (100 μg), demonstrating no toxicity after one injection. After two injections of each antibiotic spaced 48 hours apart, five of six of the gentamicin-injected eyes and three of the six amikacin-injected eyes demonstrated abnormalities of the RPE, disorganization of the outer segments of the photoreceptors, and mild loss of RPE photoreceptor interdigitation. A third injection, given 48 hours later, caused multiple white dots to appear from the posterior pole to the equator at the level of the RPE in four of nine eyes with gentamicin and vancomycin and in two of nine eyes with amikacin and vancomycin. Control eyes did not demonstrate these findings. Retinal pigment epithelial disturbance was followed by disorganization of the photoreceptor outer segments with focal disorganization of the RPE with hyperpigmentation and hypopigmentation.

Shockley and associates[72] studied ceftriaxone and found no electroretinographic or histopathologic changes after injections of 5 mg. After injection of 7.5 or 20 mg, the electroretinographic B wave was diminished but recovered after a period. There were no abnormal findings on ophthalmoscopic or histopathologic examination. After injection of 50 mg in the vitreous cavity of the rabbit, lens opacification and corneal clouding were noted transiently. There was retinal edema, and the B wave was flat at 24 hours and did not fully recover after 2 weeks. There was generalized retinal edema on histopathologic examination with disruption of the retinal layers. Schenk and associates[73] studied carbenicillin disodium and found that 10, 15, or 20 mg produced cataracts that cleared within 4 to 5 weeks.

Systemic toxicities are a significant consideration in the choice of systemic antibiotics and need to be balanced against the poor penetration ratios for many drugs now used in endophthalmitis therapy. Because of the poor penetration of drug into the eye, higher levels of doses may be given, thus increasing the risk of systemic toxicity. The three principal toxicities of aminoglycosides are nephrotoxicity, ototoxicity, and neuromuscular paral-

ysis.[74] Ototoxicity is relatively uncommon but is frequently irreversible and may occur even after the drug has been discontinued. The incidence of ototoxicity with hearing loss on audiometric testing has been reported to be 0.5% to 5% of patients given the aminoglycosides. Auditory toxicity tends to be cumulative and to be increased after repeated aminoglycoside courses, even during carefully monitored therapy. Nephrotoxicity occurs because of proximal tubular cell damage. The onset of glomerular dysfunction usually occurs several days after the beginning of therapy and increases in severity over several days. Gentamicin is thought to be more nephrotoxic than tobramycin or amikacin. Renal function needs to be monitored carefully, since renal damage is reversible and severe nephrotoxicity rarely occurs if the aminoglycoside dosage is adjusted.

Vancomycin, like the aminoglycosides, may cause neurotoxicity leading to auditory nerve damage and hearing loss.[75] Hearing occasionally improves when the drug is discontinued but more often deteriorates and becomes permanent. Nephrotoxicity is now uncommon, but serum levels should be monitored carefully when other nephrotoxic drugs are given. The most common side effects are fevers, chills and phlebitis at the site of infusion.

Cephalosporins are known to have a low level of toxic side effects, but there is a cross-reactivity with penicillin allergy. The recommendation of most authorities is to avoid cephalosporins if the patient has had an IgE-mediated reaction such as hives or has had anaphylaxis. The true risk of allergy is estimated at only 1% to 2% of individual patients.[42]

DOSE-RESPONSE ISSUES IN THE VITREOUS CAVITY

Intravitreal injection of antibiotics creates a very high concentration of drug within the vitreous cavity initially, with persistence for a variable period. The concentration and duration of exposure of the antimicrobial drug necessary for successful therapy in endophthalmitis is unknown. The MIC is the lowest concentration of an antimicrobial agent that prevents visible growth of bacteria after an 18- or 24-hour period of incubation in vitro. In antimicrobial therapy it is felt that the drug level should, at a minimum, reach a concentration that at least exceeds the MIC of the target organism. MIC values for common organisms causing endophthalmitis are given in Appendix II for reference. The MBC (minimal bactericidal concentration) is the lowest concentration of antimicrobial that totally suppresses growth on antibiotic-free media or results in a 99.9% or greater decline in colony count after overnight (18- or 21-hour) incubation. The MIC may be the same as the MBC, but the MBC may be several multiples of the MIC, in which case the organism may be said to be resistant. Research indicates that the MBC is the more critical level in the therapy of meningitis, but the MBC deter-

minations for given organisms are less readily available than the MIC levels.

Bacterial killing by antimicrobials is dependent on multiple factors, including drug concentrations, duration of exposure, site of infection, and the particular interaction between a given pathogen and a specific antimicrobial. In general, bacterial time-kill studies suggest that antimicrobials belong in one of three categories: (1) those with marked concentration-dependent activity, (2) those with time-dependent activity, or (3) those that are bacteriostatic. Aminoglycosides are characterized by concentration-dependent killing. After aminoglycoside exposure bacterial killing is rapid, and a bacteriostatic period follows when antibiotic is withdrawn. With increasing concentrations bacterial killing is more extensive and rapid, and high doses exert prolonged effects.

For beta-lactam antibiotics, bactericidal activity depends primarily on duration of exposure. Bacterial killing is slower in onset and peaks at lower concentrations. Successful therapy is related to the time that concentrations remain above the MIC, and bacteria tend to regrow when beta-lactams fall below the MIC of the pathogen. A prolonged interval before bacterial regrowth after concentrations fall below the MIC is termed the postantibiotic effect and is characteristic of concentration-dependent killing. Beta-lactams show little if any postantibiotic effect against gram-negative organisms.[76-79]

In the treatment of bacterial meningitis, there is a positive correlation between the bactericidal rate and the concentration of the antibiotic in the CSF in studies of beta-lactam treatment of pneumococcal meningitis. Unlike treatment of infections elsewhere, bacterial killing in the CSF with beta-lactams is dose-dependent but is incompletely understood.[28,29] As a general rule, in the CSF concentrations in a range slightly above the MBC are bacteriostatic or minimally bactericidal. However, large bolus doses of antibiotic are effective in meningitis treatment because the most important variable in some studies was the peak CSF antibiotic concentration. As CSF concentrations are increased to 10- to 100-fold times the MBC, the bactericidal rate increases until it plateaus at a maximal killing rate. Beyond this time, a postantibiotic effect has been noted, probably mediated by low residual levels of drug, which are even lower than the MIC. It is thus recommended in meningitis that CSF concentrations should reach levels at least ten times the MBC for effective killing. For effective killing in the vitreous cavity, these may also be desirable target levels. For beta-lactams a prolonged duration of exposure is probably also important.

Bacterial killing may have altered pharmacodynamics in endophthalmitis. Davey and associates[80] studied the rate of bacterial killing in models of experimental gram-negative endophthalmitis. While antimicrobial concen-

trations reach a maximal effective concentration in CSF and other tissues, this maximal effect was not noted in treatment of gram-negative endophthalmitis even when concentrations were 100 times the MBC. Appropriate antimicrobials given after the infection was established for 48 hours failed to eradicate the infection. Exact causes for this lack of effective killing in the model of gram-negative endophthalmitis were not clear.

Factors in the activity of antimicrobials in vivo that may make their effects different from in vitro measurements include (1) a decrease in the pH due to the infective process, which may cause aminoglycosides to lose activity, (2) slow growth rates of bacteria, which reduce the effectiveness of beta-lactam activity, and (3) reduction of bacterial doubling times by fever (ie, pneumococci in meningitis). Davey and associates[80] also hypothesized that consumption of nutrients might be a limiting factor, but they were unable to demonstrate this in their model of gram-negative endophthalmitis.

While it has been suggested by some investigators in the past that a single injection of intravitreal antibiotic in the laboratory and clinical setting may be sufficient to eradicate bacterial infections[15,81-85] it is clear that this is not universally true. *P acnes* infections, presumably the cause of slow growth rates of the organism, are notoriously resistant to single injections of antibiotic even when coupled with vitrectomy.[6] In a laboratory study of experimental *S aureus* endophthalmitis, Aguilar and colleagues[86] demonstrated that there were residual bacteria growing in 25% of the eyes after 48 hours when treatment was given either with vancomycin or with cefazolin. Gram-negative organisms and streptococcal organisms[87] have been shown to be resistant to single-dose therapy, as have even staphylococcal organisms on occasion.[88] Fungal organisms are also extremely difficult to eradicate with a single dose of intraocular medication.

SPECTRUM OF ACTIVITY

The spectrum of activity required is dictated by the common organisms causing an endophthalmitis. A broad range of gram-positive and gram-negative organisms has been identified in producing endophthalmitis. The particular distribution depends on the clinical setting. Staphylococcal organisms are predominant in essentially all series of endophthalmitis.[2-5] In postoperative cases, coagulase-negative staphylococci account for at least 50% of cases, and *S aureus* for about one third of the cases. *Bacillus* is one of the leading causes of endophthalmitis in traumatic cases.[89-91] Among gram-negative organisms, *Pseudomonas* is the most frequent cause of endophthalmitis, and *P aeruginosa* accounted for 23% of a recent series of gram-negative cases, with other *Pseudomonas* species being represented somewhat less frequently. *Haemophilus influenzae* was present in 19% of the

cases. *Proteus* species, *Serratia marcescens, Morganella morganii, Citrobacter diversus, Escherichia coli, Klebsiella pneumoniae, Enterobacter cloacae,* and *Moraxella nonliquefaciens* were also identified in this series.[92] Among the fungi, *Candida* is the most common organism encountered, with *Aspergillus* occasionally isolated, especially with intravenous drug abusers.[6]

TYPE OF ANTIMICROBIAL EFFECT

Because the eye, like the CSF, is an immune privileged site, the body's host responses to infection are somewhat different than responses to infections elsewhere.[30,31] In the brain, the blood-brain barrier excludes many macromolecules, including immunoglobulins and complement, from the CSF, creating a localized host-defense deficiency. For this reason, in the treatment of meningitis the choice of a bactericidal drug is strongly recommended and such a recommendation seems appropriate by analogy in the treatment of endophthalmitis.[20,22,23]

CEFTAZIDIME

Ceftazidime is a third-generation semisynthetic cephalosporin with promise for use in the therapy of gram-negative endophthalmitis.[42,93,94] Vancomycin is well established as the drug of choice for gram-positive endophthalmitis, since it covers essentially all staphylococcal organisms, *Streptococcus, P acnes*, and *Bacillus*.[48,49] However, vancomycin has essentially no gram-negative coverage, and an agent for initial intravitreal injection and intravenous use against gram-negative organisms is needed. Aminoglycosides have been the traditional choice for this coverage but have significant limitations because of their poor penetration after intravenous administration and significant toxicity after intraocular injection.

Studies of gram-negative endophthalmitis[92,95,96] demonstrated that almost all organisms producing gram-negative endophthalmitis were susceptible to ceftazidime and that there was no advantage in spectrum for the aminoglycosides over ceftazidime. The spectrum of activity is particularly good for *Pseudomonas*, ceftazidime being the best of the third-generation cephalosporins for *Pseudomonas* therapy. However, ceftazidime is the least active drug of the third-generation cephalosporins against gram-positive organisms, with limited activity against both *S epidermidis* and *S aureus*. Resistant gram-positive organisms include *Enterococcus faecalis* and *Clostridium difficile*. *Bacteroides fragilis* are resistant anaerobic organisms.[42]

Cephalosporins are bactericidal and interfere with the biosynthesis of the peptidoglycan component of the bacterial cell wall. The mechanism of action is to bind and inhibit specific target proteins on the inner aspect of

the bacterial cell membrane, the penicillin-binding proteins (PBPs).[42] Enzymes involved in the synthesis of the bacterial cell wall are blocked. Third-generation cephalosporins are also thought to act by inactivating endogenous inhibitors of bacterial autolysis, which disrupt the bacterial cell wall and lead to lysis of the organism.

Third-generation cephalosporins were developed for their potent activity against aerobic gram-negative rods. The basic cephalosporin molecule is a beta-lactam ring fused to a six-membered dihydrothiazine ring. The third-generation cephalosporins were developed by biochemically modifying the basic cephalosporin molecule to increase binding to bacterial penicillin protein binding sites in the cell wall. Substitution of an aminothiazole ring at the acyl side chain at C7 increases drug binding to the PBPs of gram-negative bacilli and thus enhances the spectrum of activity and potency of these drugs. Another objective in the design of third-generation cephalosporins is to decrease susceptibility to beta-lactamases and to provide longer half-lives. For ceftazidime, a propylcarboxy group has been added to increase resistance to beta-lactamases.

The third-generation cephalosporin agents must be given parenterally. Many have good penetration into the CSF and achieve sufficient levels to treat bacterial meningitis, a major advance over first- and second-generation agents.[20,22,23,42] Ceftazidime has good CSF penetration, partially related to relatively low protein binding,[43] and has been found useful in the treatment of meningitis, suggesting a possible advantage in ocular penetration. CSF concentrations of 40 μg/ml have been reported.

The dosage of ceftazidime for adult infections is 1 g intravenously (IV) or intramuscularly (IM) every 12 hours or every 8 hours, but in serious infections 2 g every 8 hours may be administered.[97] The pediatric dosage is 50 mg/kg every 8 hours. The half-life of the drug after intravenous administration is 1.9 hours, with a half-life of 2.0 hours after intramuscular injection. Peak serum concentrations of 160 μg/ml have been produced. The molecular weight of the drug is 547 ds. Ceftazidime is excreted renally, as are most third-generation cephalosporins, with 80% to 90% of the drug excreted in the urine within 24 hours.

An initial report suggests a very low risk of toxicity after intraocular administration. Campochiaro and Green[94] injected doses of 1, 2.25, and 10 mg into the vitreous of squirrel monkeys. No ophthalmoscopic or histologic changes from controls were noted with dosages of less than 10 mg. At the 10-mg dose 2 days after injection, large macular cysts were noted ophthalmoscopically and were confirmed on gross examination after enucleation. Two of the three eyes studied showed full-thickness macular holes. The outer retina demonstrated photoreceptor detachment from the RPE and dam-

age to photoreceptors with many clear cystic spaces in the outer plexiform layer of the parafoveal area. Electron microscopy of the RPE showed large, bizarre laminated inclusions with loss of microvilli and collections of amorphous materials at the apical surface. Photoreceptor outer segments were disorganized and contained irregular lamellae and large accumulations of granular material. There was disorganization of plasma membranes, and many of the outer segments were disrupted with debris in the subretinal space. Inner segments showed vacuolization, and photoreceptor damage was most marked in the macula. The inner retina and choroid were normal throughout.

Jay and associates[98] studied the ERG after injection of ceftazidime into the vitreous cavity. There was a transient B wave reduction with doses of 0.5, 1.0, 2.0, 5.0, and 10 mg, but these resolved after 7 days. Doses of 20 mg and 50 mg, however, caused a flat ERG for the entire week, which did not recover 1 week later. Tanabe and colleagues[99] tested perfused eyecup preparations and reported that A and B waves were unchanged after infusions of 0.32 mg/ml but that there was a slight suppression of oscillatory potential.

Because relatively high doses can be injected intravitreally, there is a potential for greater killing action and longer time intervals in the eye after intravitreal injection. The MIC for *Pseudomonas* for ceftazidime is 4 μg, with MIC for streptococcus and *H influenzae* at the level of 1 μg/ml or less (Appendix II).

Pharmacokinetic studies have been performed by several investigators. The serum half-life in the rabbit is 1.2 hours. Jay and colleagues[98] injected 2 mg of the drug into the vitreous of the phakic noninflamed rabbit eye and demonstrated levels of 270 μg at 48 hours and 67 μg at 72 hours. The half-life calculated from these data is 16 hours.[35] Studies of phakic eyes by Barza and associates[35] indicated a half-life of 20 hours in normal eye and 21.5 hours in eyes infected by *S aureus* after injection of 1 mg intravitreally. Elimination from the eye via the anterior route was suggested; other third-generation cephalosporins are thought to be eliminated anteriorly in some cases and posteriorly in others.

Walstad and Bilka[100] gave rabbits a single intravenous dose of 50 mg/kg. In phakic eyes there were no detectable levels after up to 5 hours, while infected eyes developed a level of 3.1 ÷ 1.2 μg/ml.[101] Walstad and associates[102] evaluated human aqueous humor penetration and found that there was a 19% penetration ratio, compared with plasma levels with average concentrations of 11 μg/ml after a 2-g IV bolus dose. Axelrod and colleagues[103] gave a 2-g IV dose and found a 4-μg maximum concentration 1 hour after intravenous injection. In rabbits aqueous levels of 7.3 μg (10% of

plasma) in noninflamed and 54 μg (16% of plasma) were found after 50 mg/kg IV doses.

Vitreous penetration after subconjunctival injection has also been studied. After 100 mg was injected subconjunctivally in the rabbit, Shockley and associates[104] identified levels of 40.2 μg/ml in the phakic and 30.5 μg/ml in the aphakic rabbit vitreous. Barza and colleagues[35] injected 100 mg subconjunctivally in normal and infected rabbit eyes. Three hours after a single injection, the normal eyes had a concentration of 7.3 1.6 μg/l; 2 hours after the last of a series of five injections given on a twice-daily schedule, the infected eyes had a concentration of 14.7 ÷ 2.0 μg/ml. Walstad and Blika[100] gave 50 mg/kg in the rabbit subconjunctivally and found levels of 15 μg/ml in the vitreous cavity. After 125-mg subconjunctival injections in human cataract patients, concentrations in the aqueous of 17 to 310 μg/ml were identified.[105] Similar subconjunctival injection doses by Barza and associates[35] resulted in vitreous levels generally less than 1 μg/ml, however. Both Shockley and associates[104] and Yannis and colleagues[106] demonstrated that subconjunctival injection prophylactically protected against the development of *Pseudomonas* endophthalmitis when bacteria were injected immediately after cataract extraction in the rabbit.

EXPERIMENTS

MATERIALS AND METHODS

Eighty-five New Zealand Albino rabbits (168 eyes) were divided into seven experimental groups. The experimental protocol was to study the ocular pharmacokinetics of ceftazidime in three anatomic conditions (phakic, aphakic, and aphakic-vitrectomized eyes). In one set of experiments, elimination of ceftazidime from the vitreous cavity following intravitreal injection was assessed (groups 1, 2, and 3) (Table IV). In a second set of experiments, penetration of ceftazidime into the vitreous cavity following intravenous administration was studied (groups 4, 5, and 6) (Table V). Ceftazidime levels

TABLE IV: CEFTAZIDIME CLEARANCE AFTER INTRAOCULAR INJECTION: EXPERIMENTAL DESIGN (VITREOUS SAMPLES OBTAINED AT EACH TIME PERIOD)

	GROUP 1: PHAKIC		GROUP 2: APHAKIC		GROUP 3: APHAKIC-VITRECTOMIZED	
HOURS	CONTROL	INFLAMED	CONTROL	INFLAMED	CONTROL	INFLAMED
2	3	3	3	5	6	7
8	4	4	4	4	6	7
24	4	4	4	4	6	7
48	4	4	4	4	8	9

APPENDIX II: IN VITRO SUSCEPTIBILITY OF VARIOUS BACTERIA CAUSING ENDOPHTHALMITIS TO SELECTED ANTIBIOTICS: MIC (μg/ml)

	CEFAZOLIN	CEFTRIAXONE	CEFTAZIDIME	CIPROFLOXACIN	GENTAMICIN	AMIKACIN	IMIPENEM
Gram-positive*							
Staphylococcus epidermidis	0.8	16.0	32.0	0.25			0.2
Staphylococcus aureus	1.0	4.0	16.0	1.0	0.8	3.1	0.1
Streptococcus pyogenes	0.1	0.03	0.25	1.0			0.1
Streptococcus pneumoniae			1.0	1.0			0.01
Enterococcus				2.0	6.2	50	0.8
Bacillus	> 100	> 64	> 64	1.0			
Gram-negative†							
Pseudomonas aeruginosa	> 100	> 32	4	0.5	3.1	12.5	12.5
Klebsiella pneumoniae	6.0	0.1	0.5	0.125	1.6	6.2	0.4
Serratia	> 100	4.0	1.0	1.0	1.6	6.2	6.3
Proteus			0.12	0.06	3.1	12.5	0.6
Enterobacter		0.1	1.0	0.125	1.6	6.2	0.4
Escherichia coli	5.0	0.1	0.5	0.03	6.2	12.5	
Haemophilus influenzae	10.0		0.1				0.1

*Minimum inhibitory concentraion (μg/ml) for 90% of strains (MIC_{90}).
†Minimum inhibitory concentration (μg/ml) for 100% of strains (MIC_{100}) except ciprofloxacin (MIC_{90}).
Adapted from Lietman,[74] Donowitz and Mandel,[113] Neu,[114] and Andriole.[115]

TABLE V: CEFTAZIDIME INTRAOCULAR PENETRATION AFTER INTRAVENOUS ADMINISTRATION: EXPERIMENTAL DESIGN (VITREOUS SAMPLES OBTAINED AT EACH TIME PERIOD)

	GROUP 4: PHAKIC		GROUP 5: APHAKIC		GROUP 6: APHAKIC-VITRECTOMIZED	
HOURS	CONTROL	INFLAMED	CONTROL	INFLAMED	CONTROL	INFLAMED
2	4	4	4	4	7	6
8	4	4	4	4		6
10					4	4
24	4	4	4	4	7	6
26					4	4
48	4	4	4	4	7	6
72					4	5

after intraocular injection supplemented by intravenous injection was studied in a final group (group 7). Each anatomic group was subdivided by creating control and inflamed subgroups. All animals were maintained in accordance with the guidelines of the Association for Research in Vision and Ophthalmology.

For all surgical procedures the animals were anesthetized by intramuscular injections of a 50/50 mixture of xylazine hydrochloride (10 mg/kg) and ketamine hydrochloride (30 mg/kg). Retrobulbar injections of 1 cc lidocaine hydrochloride 2% were performed in each group for extended surgical manipulation. Eyes were dilated with phenylephrine 2.5% and mydriacyl 1%.

For lensectomy a limbal incision was created and a capsulorhexis was performed. Phacoemulsification was performed by introduction of an irrigating/aspiration handpiece into the limbal incision, and removal of the lens was accomplished with fragmentation and irrigation of balanced salt solution with addition of heparin, 2500 U/500 cc. The posterior capsule was opened with a bent needle. The limbal incision was closed with multiple interrupted 9-0 monofilament sutures. At the close of the procedure, gentamicin (12 μg) and dexamethasone (1.2 mg) were injected subconjunctivally. The eyes were allowed to heal for 3 weeks and were periodically evaluated for resolution of inflammation.

In eyes prepared for the aphakic-vitrectomized group, the lensectomy was carried out and the incision then closed. An infusion cannula was then introduced through the pars plana 2 mm posterior to the limbus, and incisions were made at the same distance from the limbus for insertion of the vitrectomy instrument. A core vitrectomy was performed without attempts to remove the cortical vitreous. Incisions were closed with 7-0 Vicryl suture. Periocular injections and postoperative monitoring of the eye were the same as for eyes undergoing lensectomy alone.

Inflammation was produced in one eye of each animal using injection of heat-killed *S epidermidis* as in the model described by Martin and associates[16]; in almost all cases the fellow eye was maintained as control. The number of injected organisms was as follows: (1) phakic, 10^9/0.1 ml; (2) aphakic, 10^7/0.1 ml; and (3) aphakic-vitrectomized, 10^7/0.1 ml. Eyes were observed for 24 hours following injection of the heat-killed *S epidermidis* and re-examined to ensure equivalent degrees of inflammation. Antibiotic administration began 24 hours after injection of organisms.

STUDIES OF CLEARANCE AFTER INJECTION OF CEFTAZIDIME

In the three groups of eyes studied for clearance of ceftazidime after intravitreal injection, both eyes were injected with ceftazidime, 2.25 mg in 0.1 ml normal saline, 2 mm posterior to the limbus with the needle in the mid vitreous cavity.

In the phakic and aphakic groups animals were sacrificed by intravenous or intracardiac injection of sodium pentobarbital 10% at each of the following time periods after intravitreal injection: 2, 8, 24, and 48 hours. The total number of eyes sampled in each subgroup is given in Table IV. Eyes were immediately enucleated, quick-frozen in liquid nitrogen, and the vitreous was dissected free from other ocular structures by the method of Abel.[16]

In the aphakic group animals were anesthetized by injection of xylazine and ketamine hydrochloride at the following time periods: 2, 8, 24, and 48 hours. Six eyes were in the control group, and seven eyes were in the inflamed group. A sample of 0.15 ml of fluid was removed from the central vitreous cavity by inserting a 25-gauge needle through the pars plana at each time period. To check that the repeated intervention did not unduly influence the calculation of half-life values, two additional control and two additional inflamed eyes were sampled only at the 48-hour period, giving a total of eight control and nine inflamed eyes.

STUDIES OF OCULAR PENETRATION OF INTRAVENOUS CEFTAZIDIME

Ceftazidime, 50 μg/kg, was injected intravenously into rabbits at time zero and every 8 hours thereafter for the duration of the experiment. Blood samples were obtained at 15, 30, 45, and 60 minutes, and at 7.5, 23.5, and 47.5 hours after injection.

In the phakic (group 4) and aphakic (group 5) groups, animals were sacrificed at 2, 8, 24, and 48 hours after time zero by intravenous injection of xylazine and ketamine. The number of eyes for each sampling period for each subgroup is given in Table V. The eyes were enucleated and the vitreous harvested as described previously. In the aphakic-vitrectomized group (group 6) animals were anesthetized at 2, 8, 10, 24, 26, 48, and 72

hours and 0.15 ml was removed from the vitreous cavity for analysis as described previously. One set of eyes was sampled at 2, 8, 24, 48, and 72 hours. Another set was sampled at 10 and 26 hours to reduce the artifact of repeated sampling from the same eyes.

In two animals, 2.25 mg of ceftazidime was injected into the vitreous cavity and 50 μg/kg of ceftazidime injected intravenously followed by repeated intravenous injections every 8 hours for 48 hours (group 7). After 48 hours samples were removed from the vitreous cavity as described previously.

CEFTAZIDIME ASSAY

Ceftazidime concentrations in vitreous fluid were determined by a modification of an agar well diffusion assay by using a clinical isolate of *E coli* as the test organism. The assay medium was antibiotic medium (Difco Laboratories, Detroit, MI) to which para-aminobenzoic acid (Sigma) and thymidine (Sigma) were added. Standards and controls were prepared by dissolving ceftazidime (Eli Lilly Pharmaceutical Co) in sterile 0.1 sodium phosphate buffer (pH, 7) and diluting the solutions in heat-inactivated pooled normal human serum for standards with concentrations of 12.5, 25, 50, and 100 μg/ml. For assay, 15 ml of each sample was placed in precut seeded agar wells. After inoculation of the wells with standards, controls, and samples, the microbiologic plates were allowed to prediffuse at 4°C for 1 hour before incubation at 35°C for 16 to 24 hours. Inhibition zone diameters were measured, and the serum concentration was calculated from the appropriate standard curves.

STATISTICAL ANALYSIS

The pharmacokinetics of ceftazidime elimination from vitreous was characterized assuming a one-compartment model with first-order elimination. Mean vitreous concentrations were determined for each of the six groups of rabbits at 2, 8, 24, and 48 hours. The log of the mean concentration versus time was fitted by least squares linear regression analysis for each of the six groups of rabbits. Initial concentrations, immediately following a 2.25 mg intraocular bolus, were estimated by extrapolation of the linear regression lines to time zero. Pharmacokinetic parameters were calculated as follows:

Elimination rate constant (K)=slope of the regression line

Half-life ($T\frac{1}{2}$)=$0.693/K$

Ocular Vd=intraocular dose ÷ initial vitreous concentration

RESULTS

CLEARANCE AFTER INTRAVITREAL INJECTION

The intraocular concentration of ceftazidime 2 hours after intraocular injection of 2.25 mg was significantly higher in the phakic group (control, 1346.7 + 18.4 μg/ml) than in the aphakic group (control, 900.09 + 120.8 μg/ml) or aphakic-vitrectomized group (935.5 + 294.4 μg/ml), because of the larger volume of the vitreous cavity after removal of the lens (Table VI). At this time period there was no significant difference in concentration between control and inflamed eyes (Table VI, Fig 4). The volume of distribution, however, was similar for all eyes: phakic control 1.5 ml and inflamed 1.2 ml; aphakic control 2.9 ml and inflamed 2.6 ml; aphakic-vitrectomized 1.5 ml and inflamed 3.6 ml.

In phakic control eyes (group 1) the half-life was 13.8 hours and the concentration of drug was 139 + 56.8 μg/ml at the end of 48 hours. In the phakic inflamed eyes the half-life was reduced to 10.1 hours and the concentration was 56.5 + 61.1 μg/ml at 48 hours.

In the aphakic eyes (group 2) the values were slightly reduced with a half-life of 11.8 hours for control eyes and 8.7 hours for inflamed eyes. At 48 hours the concentration was 48.5 + 38.9 μg/ml for control and 19.1 + 9.9 μg/ml for inflamed eyes.

The aphakic-vitrectomized eyes (group 3) showed significantly shorter half-life values for both inflamed eyes (5.1 hours) and control eyes (4.7 hours). At 48 hours no control eye had recordable levels of ceftazidime, including the eyes sampled on multiple occasions or those sampled only once at the 48-hour time period. In the inflamed group the value of 1.6 μg/ml was due to a value of 9.3 μg/ml in a single eye; no other eye had detectable levels.

OCULAR PENETRATION AFTER INTRAVENOUS INJECTION

The serum level 15 minutes after intravenous injection was 117 μg/ml. In both the phakic (group 4) and aphakic (group 5) control eyes, no ceftazidime was identified in the eye at any time period after intravenous administration was started (Table VII). No detectable concentrations of drug were identified in the inflamed eyes until 24 hours after intravenous drug was begun in the phakic (11.3 μg/ml) and aphakic (1.4 μg/ml) inflamed eyes. At 48 hours, concentrations were slightly decreased in phakic eyes (4.6 μg/ml) and somewhat increased in aphakic eyes (5.1 μg/ml) (Table VII).

Values in inflamed aphakic-vitrectomized eyes (group 6) 2 hours after intravenous injection were as follows: 2 hours, 35.4 μg/ml; 10 hours 17 μg/ml; and 26 hours, 21.2 μg/ml. Values immediately before intravenous

TABLE VI: MEASURED MEAN INTRAOCULAR CEFTAZIDIME CONCENTRATIONS AFTER INTRAVITREAL INJECTION ([μg/ml] ± STANDARD DEVIATION)

	GROUP 1: PHAKIC		GROUP 2: APHAKIC		GROUP 3: APHAKIC-VITRECTOMIZED	
HOURS	CONTROL	INFLAMED	CONTROL	INFLAMED	CONTROL	INFLAMED
2	1346.7 ± 18.4	1310.3 ± 403.3	900.0 ± 120.8	882.0 ± 166.3	935.5 ± 294.4	812.7 ± 315.7
8	1049.5 ± 390.9	1063.0 ± 462.6	354.5 ± 235.2	353.5 ± 268.2	453.6 ± 41.5	216.6 ± 19.2
24	399.0 ± 133.2	532.5 ± 90.4	185.6 ± 167.2	135.7 ± 153.6	60.7 ± 27.3	8.1 ± 4.4
48	139.0 ± 56.8	56.5 ± 61.1	48.5 ± 38.9	19.1 ± 9.9	0.0 ± 0.0	1.6 ± 0.0
K	0.050	0.069	0.059	0.079	0.149	0.136
T½	13.8	10.1	11.8	8.7	4.7	5.1

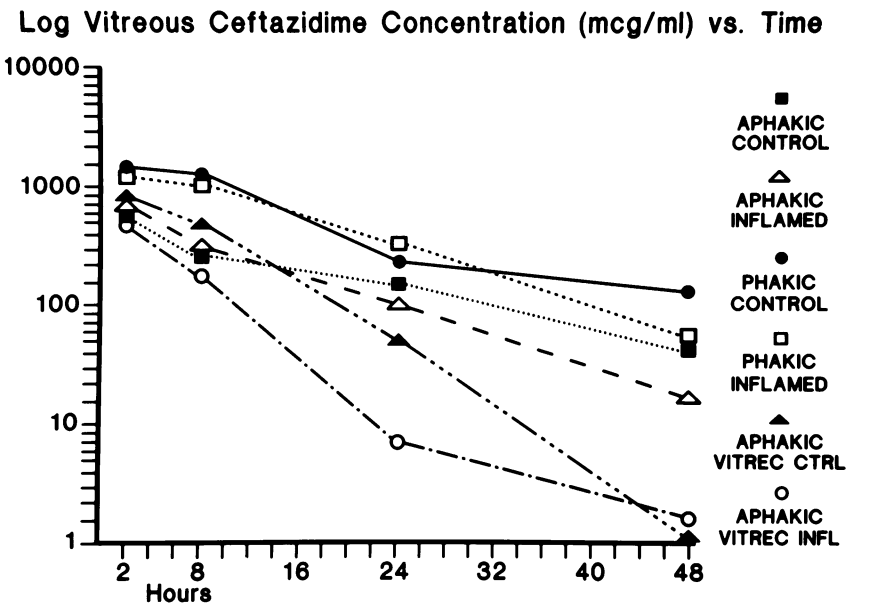

FIGURE 4
Elimination of ceftazidime from vitreous cavity, consistent with first-order kinetics.

TABLE VII: MEASURED INTRAOCULAR CEFTAZIDIME CONCENTRATIONS (μg/ml) AFTER INTRAVENOUS INJECTION

	GROUP 4: PHAKIC		GROUP 5: APHAKIC		GROUP 6: APHAKIC-VITRECTOMIZED	
HOURS	CONTROL	INFLAMED	CONTROL	INFLAMED	CONTROL	INFLAMED
2	0	0	0	0	8.5 (49%)	35.4 (18%)
8	0	0	0	0		5.4
10					12.5	17
24	0	11.3	0	1.4	2.0	9.4
26					12.0	21.2
48	0	4.6	0	5.1	8.3	10.7
72					4.9	10.1

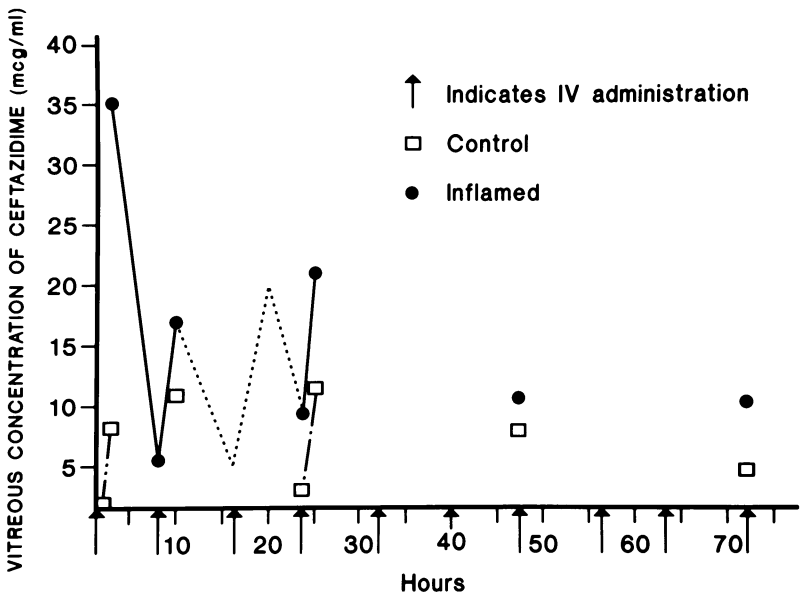

FIGURE 5
Peak and trough concentrations of ceftazidime following intravenous administration (*arrows*). Dotted line indicates estimated values.

injection were as follows: 8 hours, 5.4 μg/ml; 24 hours, 9.4 μg/ml; 48 hours, 10.7 μg/ml; and 72 hours, 10.1 μg/ml. This produced a saw-toothed pattern of concentration when data points are estimated for times not tested (Fig 5). Aphakic-vitrectomized control eyes demonstrated significant concentrations of drug at 2 hours after intravenous dosing (2 hours, 8.5 μg/ml; 10 hours, 12.5 μg/ml; and 26 hours, 12.0 μg/ml). Lower concentrations were identified immediately before intravenous injection: 24 hours, 2.0 μg/ml; 48 hours, 8.3 μg/ml; and 72 hours, 4.9 μg/ml.

In the eyes given intraocular injection and also given intravenous ceftazidime every 8 hours beginning at time zero the values at 48 hours were 6.9 μg/ml for control and 8.0 μg/ml for inflamed eyes.

Calculating penetration ratios as the average concentration of intraocular antibiotic (maximum value) divided by the serum concentration determined 15 minutes after intravenous administration gives the following values: phakic inflamed, 10.0%; aphakic inflamed, 4.5%; aphakic-vitrectomized control, 10.9%; aphakic-vitrectomized inflamed, 39%.

DISCUSSION

These studies suggest an important role for ceftazidime in the prophylaxis and treatment of endophthalmitis. There are significant advantages for ceftazidime over the aminoglycosides for both intraocular injection and intravenous administration. After intraocular injection of ceftazidime, the half-life is somewhat shorter in aphakic inflamed and aphakic-vitrectomized inflamed eyes (the usual clinical setting) than that of amikacin. This is offset by the significantly greater therapeutic range for intraocular injections of ceftazidime. Amikacin is less active in vitro than ceftazidime and thus has significantly higher MIC levels for many organisms. Because intravitreal ceftazidime has a far higher toxicity ceiling than do the aminoglycosides, the initial bolus dose may be much higher.[39,46,93]

A significant penetration of ceftazidime into the inflamed aphakic-vitrectomized eye was found 2 hours after a single-bolus intravenous dose. The average concentration at this time was 35.4 μg/ml with a penetration ratio of 39% of the peak plasma levels. These values might have been higher if tested 1 hour after the bolus dose, although aqueous concentration peaks 1 hour after intravenous administration and remains stable for between 1 and 3 hours after the bolus dose.[102] The penetration ratio is higher than the 21.5% penetration ratio reported for CSF in humans with inflamed meninges[93]; concentration levels at 10 and 26 hours are lower and the penetration ratios are more comparable to CSF penetration ratios (15% and 18%, respectively). The vitreous concentration value is significantly higher than the 7.3 μg/ml concentration reported for noninflamed aqueous levels in the rabbit but is less than the penetration ratio of 64% and concentration of 54 μg/ml in aqueous from the inflamed rabbit eyes.[101] This concentration of ceftazidime achieved in inflamed aphakic-vitrectomized eyes is therapeutically significant; it is nine times the average MIC for *P aeruginosa* and greater than 30 times the MIC for *Streptococcus, Klebsiella, Serratia, E coli*, and *H influenzae*.[93] While a single intravenous dose of ceftazidime can achieve intravitreal concentrations which should be sufficient for bacterial killing in the inflamed aphakic-vitrectomized eye. Intravenous amikacin does not penetrate sufficiently for bacterial killing in the inflamed aphakic-vitrectomized eye, intravenous amikacin does not penetrate sufficiently under the same conditions to reach its MIC for *P aeruginosa*. Gentamicin administered intravenously has not been shown to reach intraocular therapeutic concentrations in human or animal studies with the vitreous intact.[44,50,51]

Although bacterial killing studies have not been done for ceftazidime in the treatment of endophthalmitis, beta-lactam antibiotics in general need

prolonged levels above the MIC for effective action. An initial dose of 2.25 mg has not produced toxic changes in the monkey[94] and appears to be a safe intravitreal dose of humans, although clinical experience is limited. A dose of 2 mg may be chosen to make the dilution procedure easier. Supplementation by intravenous administration seems to be indicated, especially after vitrectomy when the half-life of the drug is short. Usual doses are 1 to 2 g every 8 hours in adults; the higher dose will produce a higher serum level, which favors movement across the blood-ocular barriers.

After injection of ceftazidime into the vitreous cavity of phakic control eyes, the half-life of 13.8 hours is twice as long as cefazolin (6.5 hours)[16] but only half as long as amikacin (25.5 hours).[18] Since this value is intermediate between the usual values for anteriorly removed drugs and those eliminated by a posterior route, both routes of removal may be involved. Reduction of the half-life by removing the lens suggests an increased role for anterior elimination in the aphakic eye. Inflammation decreases the half-life, apparently by increasing outward permeability. Active transport is probably not a significant factor in removal of ceftazidime, since then the half-life would be more similar to that of cefazolin, and the half-life would be increased by inflammation.

Vitreous removal has the most important effect on clearance after intraocular injection. Compared with phakic control eyes, the half-life of drug in aphakic vitrectomized eyes is only 35% as long. Comparing phakic inflamed eyes with aphakic-vitrectomized inflamed eyes, vitrectomy decreases the half-life by 50%. The effects of vitreous removal are so great that there is essentially no difference in half-life between inflamed and control eyes.

The pattern of peak and trough demonstrated in the vitreous after intravenous injection gives a sawtooth appearance to graphed values from the inflamed eye consistent with increased penetration across the blood-ocular barriers and short half-life with rapid elimination from the vitreous cavity. This pattern of penetration into the vitreous cavity is different from that of cefazolin. Cefazolin concentrations demonstrated a slow build-up with increasingly higher levels over 49 hours of testing, although only the peak levels not the trough levels were evaluated. For cefazolin, the concentration at 49 hours was three times as high as the concentration 1 hour after the initial injection.[16] The penetration ratio after the initial dose was, therefore, 4%, rising to 15.4% after 49 hours, demonstrating considerably greater permeability to ceftazidime in the inflamed rabbit eye compared with cefazolin.

In the aphakic vitrectomized control eyes, there was an apparent similar saw-toothed pattern to concentrations, although the range of values from trough to peak was smaller, indicating less permeability than in the inflamed

eye. These findings were consistent with the increase in concentration secondary to inflammation noted in aqueous humor levels, where concentrations were almost eight times higher in inflamed eyes than in controls.[101] The vitreous cavity concentrations achieved in the control eyes were above the MIC for *Pseudomonas* but only by a factor of 2, while they were eight times the MIC for other significant gram-negative organisms.

The effects of the inflammation were also noted in phakic and aphakic eyes without vitrectomy. There was no penetration into eyes without inflammation, consistent with the findings of Walstad.[100] Even with inflammation, significant concentrations of drug did not appear in the vitreous until 24 hours after the first intravenous bolus dose. In the phakic eye the levels exceeded the MIC for *Pseudomonas* by a factor of 3 and other gram-negative organisms by a factor of 10 at the 24-hour sampling time. Forty-eight hours after the beginning of the drug administration, the concentrations were essentially equivalent in aphakic and phakic eyes, with levels of 5.1 μg/ml and 4.6 μg/ml, respectively.

There was drug penetration into the vitrectomized control eyes but not into the phakic or aphakic control eyes, further underscoring the idea that vitreous is a barrier to permeability. These low levels call into question the role of intravenous ceftazidime in the prophylaxis of infection in the vitreous cavity. Although concentrations do exceed the MIC of most gram-negative bacteria by 24 hours, organisms are probably in a phase of rapid replication early after their introduction into the vitreous cavity when drug levels are relatively low or undetectable. On the other hand, significant concentrations following intravenous administration have been identified in the aqueous humor,[100,102] indicating a role in intravenous administration in the treatment of bleb infections or for prophylaxis of anterior-segment wounds.

When intravenous administration is begun after an intraocular injection, it is unlikely that there is significant penetration into the vitreous cavity from plasma during the initial phases of intraocular drug clearance. During most of the early phase of the intraocular elimination, the intraocular concentration is higher than the plasma concentration, so no concentration gradient is present. Therefore, unless active transport from plasma is involved there is not a direct contribution from plasma to the intraocular concentration. For phakic and aphakic inflamed eyes vitreous levels remain above peak plasma levels until 18 to 36 hours after intravitreal injection; in aphakic-vitrectomized eyes, they remain at this level for 10 to 12 hours.

The intravenous dose may prolong the time during which concentrations stay at or above 35 μg/ml in inflamed aphakic-vitrectomized eyes. The relatively high plasma concentration establishes a gradient favoring penetration into the eye as the intravitreal concentration falls. Without active

transport from the eye, however, the concentration gradient will not favor clearance from the eye and should prolong high levels. This effect on concentrations is short-lived, and the concentrations 48 hours after intraocular administration supplemented by intravenous administration were the same as those identified after intravitreal administration only.

If the target levels for treatment of intraocular infection are ten times the MBC for invading microorganisms (as for meningitis), we may use the MIC for rough approximation of desired concentration levels since MBC levels are not commonly available clinically. For ceftazidime the target level is then 40 μg/ml for *P aeruginosa*, 10 μg/ml for *Serratia*, 5 μg/ml for *E coli* and *Klebsiella*, and 1 μg/ml for *H influenzae*. Estimating concentration levels after an injection of 2.25 mg of ceftazidime into the human vitreous cavity with a 4-cc volume gives an initial concentration of 562 μg/ml. The concentration will fall to 35 μg/ml at the end of four half-lives. If Maurice is correct that the half-life in the human is 1.7 times that of the rabbit,[26,36] four half-lives for the human inflamed phakic eye are 68 hours, for the inflamed aphakic eye 61 hours, and for the inflamed aphakic-vitrectomized eye 35 hours. The concentrations will remain at or above the desired levels for gram negative organisms other than *P aeruginosa* for an additional two half-lives, extending the effective duration of a single intraocular injection by 50%.

Because the total duration of effective concentrations may not be sufficiently long to sterilize the vitreous cavity, particularly in the vitrectomized eye, supplementation by intravenous administration may be important. The data on penetration into the inflamed eye, particularly after vitrectomy and lensectomy, demonstrate a definite advantage for ceftazidime over the aminoglycosides. While a single dose of ceftazidime can achieve concentrations roughly equal to nine times the MIC of *P aeruginosa* in the inflamed aphakic-vitectomized eye, intravenous amikacin does not penetrate sufficiently under the same conditions to reach its MIC for *P aeruginosa.* Amikacin is less active in vitro than ceftazidime and thus has significantly higher levels for MIC. Of even more importance is the significantly lower absolute concentrations attained because of the poorer penetration of aminoglycosides even into the inflamed eye.

An important caveat in extrapolation of these studies directly to application in treatment of human disease is that the differences in pharmacokinetics between the rabbit model and the human remain unexplored. Protein-binding of ceftazidime is different in the rabbit and the human. The size of the vitreous cavity and differences in aqueous flow are also potential sources of deviation from theoretical calculations. Perhaps of greatest importance is the difference between the retinal vasculature of the rabbit and human,

since the human retina is significantly more vascularized. This may affect both clearance and penetration because there is a broader area for exchange. In comparison of clearance values between monkey and rabbit, however, there is close agreement in half-life values for gentamicin[39,41] and for cefazolin.[16,41]

These experiments emphasize the importance of studying pharmacokinetics in inflamed eyes and in eyes with the vitreous removed. Intravitreal antibiotics are almost always injected into inflamed eyes, and intravenous administration of antibiotics is usually begun in the clinical setting of infection or inflammation. Negative data about penetration of drugs into the intact vitreous of eyes in which blood-ocular barriers are not disrupted are limited in value. Antimicrobial treatment of endophthalmitis is administered in conjunction with vitrectomy in the majority of clinical cases. Because penetration and clearance of drugs in eyes after vitrectomy is so different from that in intact eyes, studies of antimicrobial pharmacokinetics should include eyes that have undergone vitrectomy and lensectomy in order to reach meaningful clinical conclusions. Preparing and maintaining the animals for these studies is time-consuming, labor-intensive, and expensive, but is necessary to give potentially useful data.

SUMMARY AND CONCLUSIONS

Ceftazidime has pharmacokinetic advantages for treatment of endophthalmitis caused by gram negative-organisms by intravenous administration. Additionally, its spectrum of coverage for these organisms and its relatively low toxicity after intraocular injection are favorable attributes. These studies demonstrate that inflammation leads to a significant reduction of the blood-ocular barriers to ceftazidime. This increased permeability shortens the half-life of the drug after intraocular injection but allows a significant penetration into the eye after a single intravenous dose so that therapeutic levels are achieved. Ceftazidime appears to be removed by both the anterior and the posterior route without active transport. The experiments demonstrate the importance of the vitreous as a barrier to achieving significant concentration of antibiotic within the eye after intravenous administration and confirm the importance of the vitreous in prolonging the half-life of drugs injected intravitreally. Finally the results emphasize that the pharmacokinetic behavior of drugs for treatment of endophthalmitis must be assessed in inflamed eyes both with and without intact vitreous, since these factors play a large role in drug availability and concentration in the vitreous cavity and are the major variables in the clinical setting.

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