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OBSTRUCTION AND APPROACHES TO CROSS THE DRUG MOLECULES THROUGH BLOOD RETINAL BARRIER: AN OVERVIEW

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ABSTRACT

It is a challenging task to administer the appropriate quantities of drugs to the eye mainly to the retina. Retinal transmission is urgently required due to potential vision loss caused by retinal disease. The failure to provide retinal transmission of topical or systemic routes is now widely accepted. The intravitreal path offers a high regional density of drugs which induces cataracts, retinal detachment, and endophthalmitis. Periocular route utilizing the sclera's permeability for the transmission of retinal dug. Systemically administered drugs must clear the retinal blood barrier (BRB) to show the action. The internal and outward flow of drugs is carefully regulated by highly specialized ocular barriers. A better understanding of these biological barriers could lead to new developments in ophthalmic drug therapy, including customised administration and minimally harmful side effects. This study primarily investigated the anatomical structure of the eye, specifically the blood-retinal barrier (BRB), different methods of drug administration, the importance of BRB physiology, such as its barrier functions, and the impact of influx and efflux transporters on delivering medications to the retina.

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Introduction

Partitioned into two main portions, the anterior and posterior, the eye is one of the body's most delicate organs. The rear or posterior region makes up the two-thirds of the eye. Encircling the vitreous organ, an inner chamber filled with gel, are three main layers: the choroid, retina, and sclera [1], the anatomy of the eye is depicted in **Figure 1**. The sclera gives the eyeball structural stability. Its interior is closely related to the choroid, a highly vascularized surface that supplies internal retinal feeding and clearance. The extremely intricate neurosensory membranes of the retina are eventually found in the innermost layer of the back segment. The retina is a complicated structure with two layers of synaptic strands, three neuronal layers, and one epithelial layer, with a thickness that can reach several hundred microns [2]. The retina is composed of neural cells and glial cells, including Muller cells, astrocytes, microglial cells, and oligodendroglial cells [3]. The retinal pigment epithelium (RPE) is a layer of pigmented cuboidal epithelial cells that covers the outermost section of the retina. It plays a role in immunological regulation, light absorption, phagocytosis, and substrate transport into and out of the retina [4, 5]. The photoreceptor cells in the RPE have rods and cones that play a crucial role in absorbing and converting light photons into biochemical signals. As the visual signal travels, it passes through different types of cells, such as bipolar cells for cones and both bipolar and amacrine cells for rods. Similar to a biologist, one can observe that the ganglion cells, which comprise the majority of the retina's neurons, play a crucial role in transmitting visual information from the retina to the brain through the closely spaced optic nerve. Similar to a biologist, Muller cells play a crucial role in providing structural support and surrounding the dendrites of retinal cells. They also have the important task of regulating levels of extracellular potassium, g-aminobutyric acid, and glutamate [6-8]. The gel structure that fills the vitreous body, known as vitreous humour (~ 4 mL), is composed of hyaluronic acid (HA), proteoglycans of chondroitin sulphate and heparan sulphate, collagens (types I, V, IX, XI), non-collagenous proteins (fibrillin-1, opticin, VIT1), and liquid (99%). Humour possesses structural characteristics that

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are derived from its various components [9]. Two important functions of humour are to maintain the health of the eyes and to move nutrients to and from the retina [10]. The retina's structural details are seen in **Figure 2**.

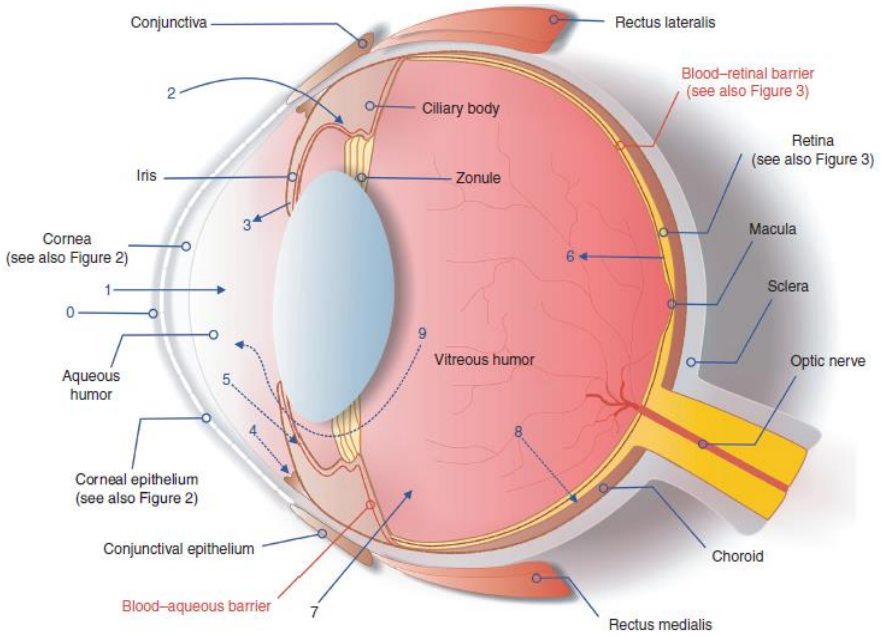


Figure 1. Structure and biological barriers of the eye

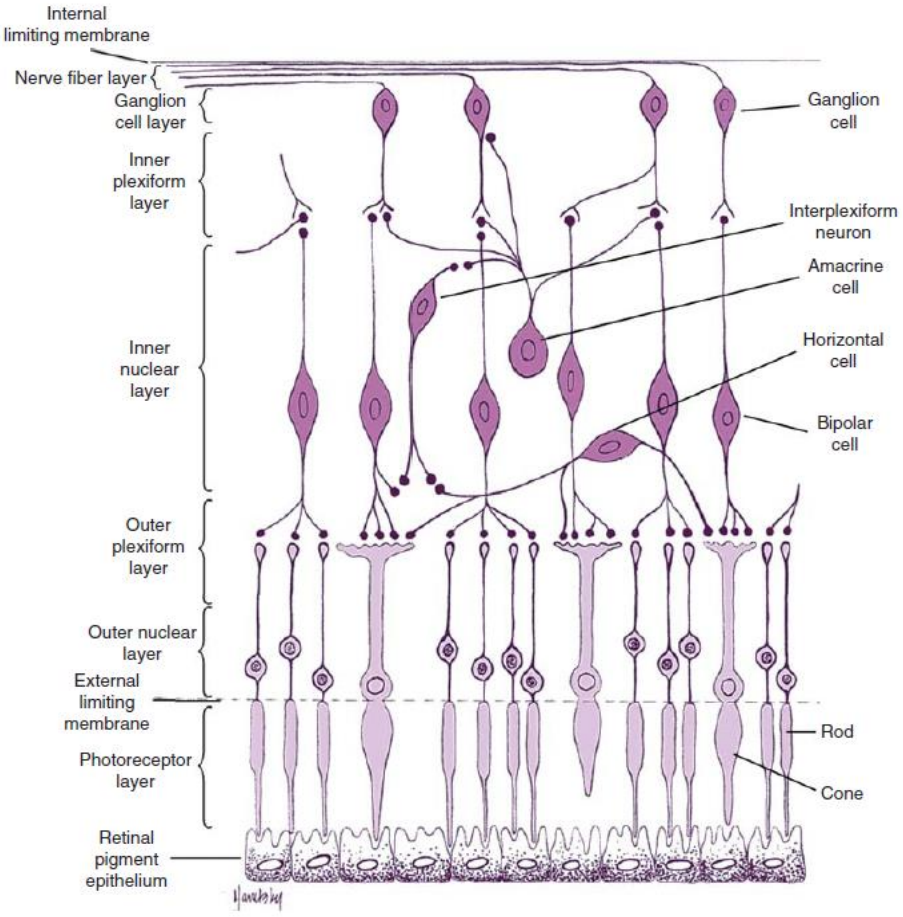


Figure 2. Anatomy of retina

Problems of the Retina

The vision is affected by retinal disorders and some may be serious enough to cause blindness. They are

- *Macular Degeneration:* This condition affects both sharp and central vision. There are various factors that can contribute to the cause, including genetic, eye-specific, and systemic disorders like diabetes and high blood pressure. Choroidal neovascularization (CNV) occurs when new blood vessels from the choroid invade the retina. This process is caused by long-term oxidative damage and the fenestration of the retinal pigment epithelium (RPE) [11].
- *Diabetic Eye Disease:* This results in bleeding of the blood vessels in the retina as well as damage to various cellular structures in the retina due to osmotic and inflammatory effects [12].
- *Glaucoma:* This occurs as different unrelated pathologies that cause a significant proportion of ganglion cells to degenerate. A class of eye conditions known as glaucoma harms the optic nerve, which is necessary for clear vision. It also damages your eyes when the pressure inside them is abnormally high. It is one of the main reasons why people over 60 go blind [13].
- *Retinal Detachment:* When the retina becomes detached from the back of the eye, it is considered a medical emergency.
- *Retinoblastoma:* It is a cancer of the retina and is common in young children.
- *Macular Pucker:* It is the scar tissue of the macula.
- *Hole of Macular:* A small break in the macula which is common in people above 60 years.
- *Floaters*: Cobwebs or specks in the field of vision

WHO has ranked Diabetic retinopathy (DR), glaucoma, and age-related macular degeneration (AMD) among the top 10 priority eye diseases. Retina-targeting glaucoma treatments are currently unavailable, with primary management for this condition being eye anti-hypertensive. DR and late-stage AMD are regulated by the vascular endothelial growth factor (VEGF) and therefore treatment focuses on preventing this mediator's actions by using anti-VEGF antibodies (ranibizumab, bevacizumab) administered intravitreally [14, 15]. It's significant to note that none of the three diseases have a known cure as of yet; instead, continuous, intensive therapy is required. Retinitis pigmentosa, retinitis caused by the cytomegalovirus (CMV), uveitis, and occlusions of the retinal vein and artery are a few of the primary conditions that affect the eyes.

Routes for Retinal Drug Administration

The treatment may be delivered to the retina using five regularly utilized routes of administration, such as systemic (oral or intravenous), topical, periocular, suprachoroidal, and intravitreal routes, as shown in **Figure 3**. Although advantageous since they are simple to deliver, limited bioavailability (less than 5% of the supplied treatment can penetrate the retina) is a problem with both topical and systemic routes. Above all, neither route works well for administering large-molecule medications like gene therapy or antibodies, which are the most likely treatment options [16].

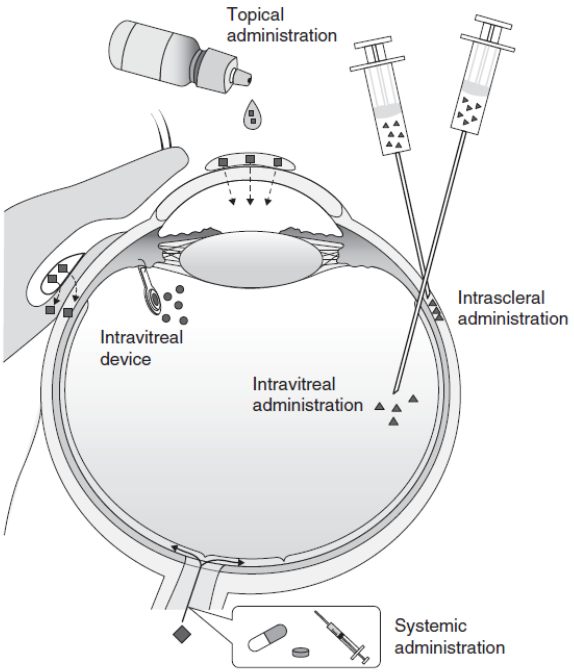


Figure 3. Routes of drug delivery for retinal diseases

The administration of various medications, such as steroid therapy triamcinolone, has now become a well-established clinical procedure by periocular delivery, which entails putting drugs as depots in areas surrounding the eye. Iontophoresis large macromolecules like bevacizumab (~150 kDa) have been demonstrated to traverse successfully, indicating the technique's enormous potential. Since the treatment just needs to pass through the choroid and sclera to reach its goal, the periocular route may also be thought of as the most efficient way to deliver medication to basal retinal tissue. The periodic path includes subconjunctive, subtenon, retrobulbar, peribulbar, and subsequent juxtascleral drug delivery methods to the retina. Depending on its concentration and barrier characteristics, medication can be transported to the choroid, neural retina, retinal pigment epithelium, sclera, and vitreous if it is taken via these pathways. A beveled edge needle with a maximum gauge of 25–30, a length of 30 mm, and a capacity of up to 0.5 ml is used for subconjunctival injections. A 2.5 cm long blunt-tipped cannula needle is inserted into the tenon's capsule during sub-tenon surgery, and up to 4 milliliters of medication are injected. A 26 gauge 5/8 inch needle with a sharp tip is helpful for posterior sub-tenon injections. Intraocular injection in the conical area behind the cone globe of the eye is called retrobulbar. 25–27-gauge blunt needles are utilized for this injection. A 1.25-inch needle and 8–10 milliliters of the anesthetic are used in the peribulbar 25 gauge. Typically, 0.5 milliliters of the drug are injected gradually throughout the posterior juxta scleral delivery [17]. **Figure 4** shows the drug routes following periocular delivery. Nonetheless, several physical barriers (retinal barriers, endothelial blood vessel cells) and biochemical barriers (conjunctive absorption, lymphatic clearance, rapid efflux by active transporters, enzymatic degradation) remain related with this path, unfavorably affecting drug bioavailability. Since spreading across the sclera, there are several obstacles that the drug can encounter. Following the sclera, the following tissues are identified by the medication: choroid, retinal pigment epithelium, neural retina, vitreous membrane, and inner limiting membrane, in that order. Depending on the intended location, the medications must pass through one or more periocular surfaces. Many of these could provide major obstacles to the administration of retinal medications, with retinal pigment epithelium and choroidal blood flow posing the most obstacles. The circulatory supply in the choroid is probably going to remove the medication fast [18].

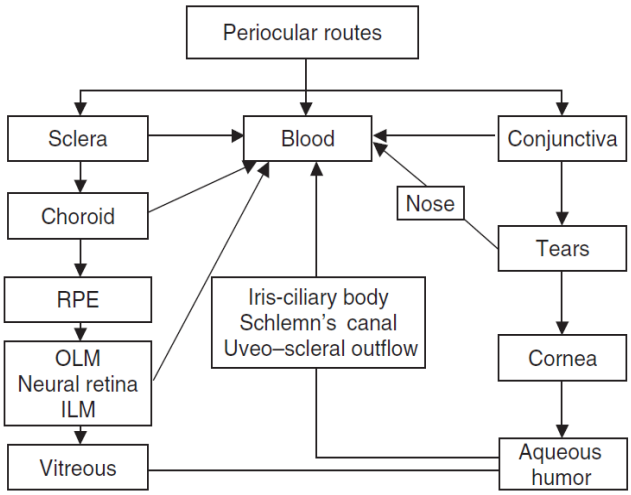


Figure 4. Pathways of drugs after periocular administration

Drug administration has reportedly been made easier by the development of microneedle technology, which inserts the needle straight into the suprachoroidal space—a area that sits between the choroid and the sclera. Between the intravitreal and periocular channels, the suprachoroidal pathway—which is extremely minimally invasive and circumvents all restrictions related to sclera diffusion—might prove to be a helpful intermediary. Suprachoroidal injections may be able to deliver a substance to retinal tissue over an extended period of time; however, other substances—like steroids and b-blockers—have been shown to enter the retina efficiently via this route [19, 20].

The most popular and efficient method of treating the eye is intravitreal administration, which involves injecting or inserting formulations into the vitreous humour. When given, the medication can be concentrated at the target area and remain there, lowering overall toxicity. This approach's low patient acceptability—caused by the pain and irritation of repeated, lengthy vitreous injections—is a major drawback. It's also crucial to consider the restricted amount of therapy that can be given intravitreally, with a 100 µL injection volume being the suggested maximum. Many major eye problems are frequently related to this administration route, comprising endophthalmitis, retinal detachment, cataract development, vitreous hemorrhage, hyphema, uveitis, loss of visual acuity, and elevated intraocular pressure [21]. Drugs cannot pass through the inner limiting membrane (ILM) to reach the retina; it is located at the interface between the venomous humor and the retina. The intercalarymal membrane (ILM) has a striking resemblance to other basement membranes. It is comprised of a type IV collagen, proteoglycan, and laminin film that is attached to the outer membrane of Muller glial cells [22]. With the exception of the optical disc, the barrier completely encloses the retina's surface. In the foetal stage, its thickness is approximately 70 nm, and by late adulthood, it has increased to well over 1 µm. There is a notable wide inter-individual variation in ILM

thickness, with major increases in membrane observed in specific disease conditions (e.g., DR). Age-related changes in the metabolic makeup of ILM cause an increase in membrane stiffness [23].

Blood Retina Barrier (BRB)

Figure 5 illustrates that the blood-retinal barrier (BRB) consists of endothelial retinal capillary cells (inner BRB) and retinal pigment epithelium (RPE) cells (outer BRB) that are tightly interconnected. The functional barrier provided by this mechanism restricts the passage of non-specific substances between the circulating blood and the neuronal retina [24, 25]. While the exact function of BRB in aiding the retina is evident, a significant obstacle for retinal drug treatment in general is the limited ability of medications to enter the retina from the bloodstream. The inner two-thirds of the human retina are nourished by the inner blood-retinal barrier (BRB), while the outside BRB supplies choriocapillaris to the rest of the retinal tissue [26, 27]. Therefore, the necessary nutrients for photoreceptor cells are distributed along the outside border of the blood vessel (BRB), whereas the majority of the nutrients needed for neuronal cells, such as ganglion cells, bipolar cells, horizontal cells, amacrine cells, and Muller cells, are obtained via the inner BRB. The formation of cohesive monolayers with strong interconnections between retinal capillary endothelial cells and RPE cells hinders the unrestricted movement of substances between the circulating blood and the neural retina via the gaps between cells. As an example, the permeability of D-mannitol, a paracellular marker that cannot pass through cell membranes, is more than 190 times lower than the permeability of D-glucose and L-arginine, which are transported across cell membranes. The monolayers are completely polarized since the transporters in the separate membranes of the retinal capillary endothelial cells (RPE) are accountable for transporting both metabolic waste products and nutritional substrates from the blood to the retina. The Na+, K+-ATPase is mostly located in the apical region of polarized RPE cells, where it regulates the balance of intracellular Na+ and K+ ions. The luminal membrane of retinal capillary endothelial cells is in direct contact with the retina, whereas the abluminal membrane is in contact with the blood. Similarly, the apical and basolateral membranes of RPE cells come into contact with the retina and choroidal blood, respectively [28, 29].

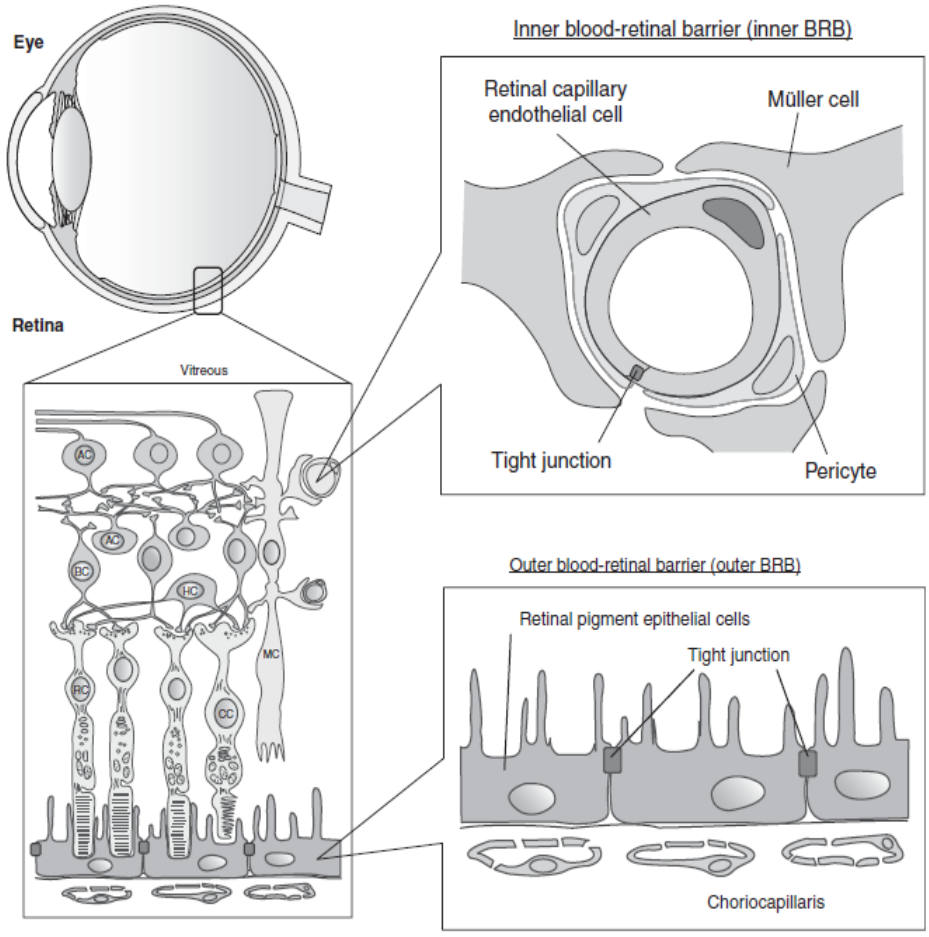


Figure 5. Diagram of blood-retinal barrier

Uptake Index of Retina/ Retina Uptake Index

Alm and Teornquist first documented the retinal uptake index (RUI) based on the available tissue sampling-single injection methods, which is a modification of the brain uptake index (BUI) and the RUI received significant *in vivo* data on the BRB-

to-retina transportation process [30]. The advantage of using the carotid artery injection method is that it eliminates the impact of plasma-protein binding on the test substrate and allows for the examination of retinal absorption in the presence of an unlabeled competitor. This is possible because only a small portion (less than 5 percent) of the injected bolus (approximately 200 µl) is mixed with the plasma [31]. In this technique, the carotid artery is rapidly inserted into a small bolus containing a trace dose of the (^{3}H) marked compound of interest and a highly diffusible reference compound (^{14}C) butanol or (^{3}H) water while testing a sample compound (^{14}C). Upon injection, the animal (usually a rat) is decapitated for a short time (typically 15 sec) and tissue and injection solution specimens are analyzed by the scintillation counting method [32]. The calculation of the RUI is as follows:

$$RUI = (([3H]/[14C])\ (\text{dpm in the retina}))/(([3H]/[14C])\ (\text{dpm in the injection solution}))\ \times 100 \quad (1)$$

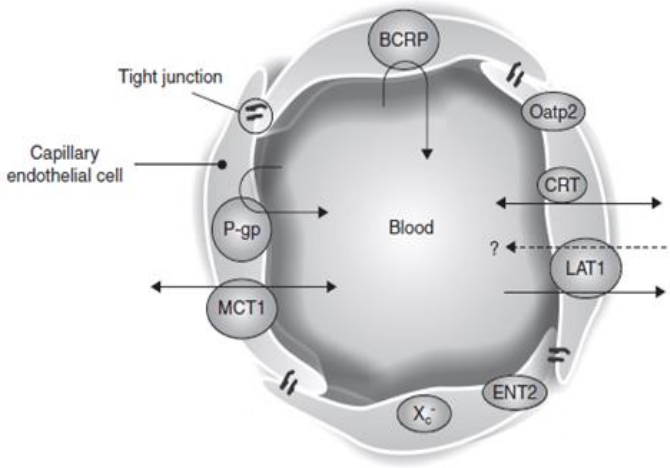
The RUI technique was used primarily to determine permeation under appropriate sink conditions and is especially useful to determine the effect of physicochemical parameters on initial retinal uptake. The RUI approach can be used to assess if a carrier is involved in drug retinal absorption across the BRB. A close relationship between the RUI and lipophilicity for a variety of chemical groups was developed by the use of thirteen compounds predicted to be transfered from blood to the retina through passive diffusion and with a log n-octanol / Ringer distribution coefficient (DC) ranging from-2.6 to 2.5. Toda *et al.* recently reported a similar relationship [33]. While compounds that do not display significant influx and efflux transport exhibit a predictable relationship between RUI and DC, several compounds known or suspected to be influx transporter substrates have significantly higher RUI values than those that would be predicted based solely on their lipophilicity.

Strategy to Improve the Retinal Penetration

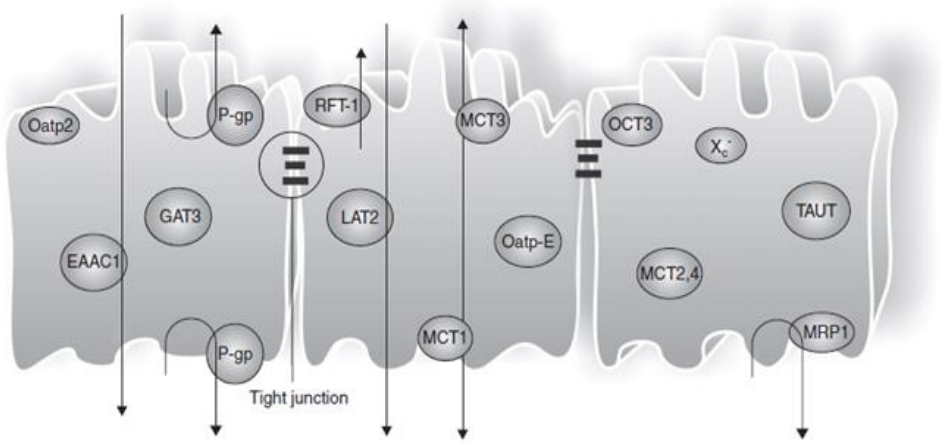
- *Particle Targeting*: The surface charge and structure of nanoparticles considerably affect their capacity to enter the retina, as shown by the time-dependent mobility of several self-assembled nanoparticles in rat eyes [34]. These findings align with previous studies indicating that cationic particles had difficulties entering the retina. Specifically, polyethyleneimine nanoparticles clustered together at the chitosan vitreous, whereas glycol particles landed on the ILM without breaching the barrier. On the other hand, human serum albumin and anionic HA-based nanoparticles penetrated retinal tissue significantly more deeply. When nanoparticles successfully penetrate the retina, they can stay there for months, acting as enticing stand-ins for surgical implants. Rat retinal pigment epithelial cells (RPE cells) were where the PLA nanoparticles were found 24 hours after injection and remained for up to 4 months [35].
  Compared to native medicines, liposomal formulations have demonstrated superior pharmacokinetic characteristics over a broad spectrum of clinical conditions. They have no trouble penetrating the retina. Tacrolimus-containing liposomes, for instance, were injected intravitreally and associated with rat ILM in six hours, with a significant accumulation of the outer nuclear layer occurring in twenty-four hours. Presence in the retina has been observed for twenty-one days, indicating that liposomes, as opposed to aqueous medications, encourage the achievement of greater and more stable regional concentrations [36]. An essential alteration to reduce the premature elimination of nanoparticles is attaching specific moieties to their surface by conjugation. While ligand conjugation may potentially be achieved with any kind of carrier, liposomal systems have shown the highest degree of success and comprehensiveness. The utilization of multiple homing peptides, such as YSA (which specifically binds to the neovascular receptor Ephrin A2), RGD (which targets rapidly dividing endothelial cells), ATWLPPR (which specifically binds to VEGFR-2), and APRPG (which targets angiogenic vessels), has enhanced the efficiency of liposomes in delivering their contents to rat CNV in vivo. The prognosis for each instance has markedly improved as a consequence of this [37-39].
- *Viral Vectors*: The ability of viral vectors to safely protect genetic material, use their stealth to cross biological membranes, and effectively target different cell receptors through a variety of binding proteins on their surface has made them a popular method for delivering genes for many years [40]. To date, from a retinal gene delivery perspective, AAV vectors remain the most successful [41]. Sadly, there are several problems with viral vector technology, including difficulties in producing large quantities, insufficient ILM penetration leading to low intravitreal efficacy, and high immunogenic potential. Nonetheless, some progress has been made in enhancing the immunogenicity of these vectors [42, 43]. Considering this, a revised AAV vector has shown enhanced in vivo gene transfection in the rd12 animal model of Leber's congenital amaurosis, a rare hereditary retinal disease. This has allowed the therapeutic gene cargo (RPE65) to successfully restore function and provide significant visual improvements [44].
- *Sonotherapy*: Several stimulus-triggered targeting techniques are still in the early phases of research, however drug targeting for sonotherapy is becoming more popular as a means of delivering intravitreal retinal medication. The non-invasive application of ultrasonography to improve drug penetration through diverse biological membranes and cells is known as sonotherapy [45, 46]. In a medium known as cavitation, ultrasound promotes the nucleation, development, and oscillation of gaseous particles [47]. Under the correct conditions, ultrasonography can burst gas bubbles and produce the energy required to momentarily increase blood vessel and cell membrane porosity, which promotes drug transport and deposition in the surrounding tissue [48]. Co-administration of PEGylated fluorescent polystyrene nanospheres and ultrasonic ex vivo via the retina of cows produced notable enhancements in retinal formulation permeability, enabling increased absorption of RPE cells within the nanosphere [49, 50].

Blood-To-Retina Influx Transport of Drugs

The permeability of the membrane is a crucial determinant of pharmacokinetic behavior, such as drug absorption, distribution, metabolism, and excretion (ADME). A medication must cross the BRB by passive diffusion and/or transporter-mediated transport to induce its pharmacological and therapeutic effects. The known transporters through the BRB are depicted in **Figure 6**.



a)



b)

Figure 6. Known transport mechanism in the retina

- *Nutrients:* Self-inhibition was detected in RUI investigations, indicating that nutrients have a permeability rate that is up to 4000 times greater than that of D-mannitol, a non-permeable paracellular marker. This suggests the presence of specialized transporters responsible for the transfer of these substances from the blood to the retina. Specialized transporters enable the movement of several nutrients, including hexose, amino acids, monocarboxylic acids, nucleosides, amines, and vitamins. Facilitative glucose transporter 1 (GLUT1/SLC2A1) transports D-glucose and dehydroascorbic acid (DHA) from the circulation to the retina. These substances act as the primary sources of energy for the retina and contribute to the presence of oxidized vitamin C [29, 51]. GLUT1 is found in the brush and basolateral membranes of the outer blood-retinal barrier (BRB), as well as in the luminal and abluminal membranes of the inner BRB [52]. While GLUT1 is a facilitative transporter expressed on both sides of the membranes, the transportation of D-glucose and DHA from blood to the retina predominates in the opposite direction rather than in the opposite direction.
- *Amino Acid-Mimetic Drugs:* L-leucine and L-phenylalanine serve as precursors for neurotransmitters and protein synthesis. They are delivered from the blood to the retina by a Na+-independent amino acid transporter known as system L at the blood-retinal barrier (BRB) [53, 54]. System L also contains amino acid mimetic medicines, including L-dopa (a precursor of dopamine), gabapentin (an analog of g-aminobutyrate), and mustard amino acids, owing to its high substrate selectivity [55, 56]. System L is encoded by the amino acid transporter SLC7A5, also known as LAT1, and the amino acid transporter SLC7A8, also known as LAT2. LAT1 and LAT2 have a distinctive characteristic in that they need an extra protein, namely the heavy chain of the 4F2 cell surface antigen (CD98/SLC3A2), for their functional expression. An immune histochemistry investigation revealed that LAT1 is mostly expressed in endothelial cells of

retinal capillaries [53]. While RPE cells express mRNA for LAT1 and LAT2, quantitative RT-PCR and functional analysis using ARPE-19 cells indicate that LAT1 contributes significantly to the uptake of L-leucine [51].

- *Monocarboxylic Drugs:* Monocarboxylates, like lactate, pyruvate, and ketone bodies (b-hydroxybutyrate and acetoacetate), play a key role in most mammalian cell physiology. L-Lactic acid, in general, is produced as an end product of glycolysis in enormous quantities. More L-lactic acid is aerobically developed in the retina than in any other tissue [57]. Furthermore, it seems that L-lactic acid is also needed by photoreceptors as an energy source in addition to D-glucose. The SLC16 family of monocarboxylate transporters (MCTs) is composed of H+-coupled MCTs, while the SLC5 family of monocarboxylate transportation transporters (SMCTs) includes some monocarboxylate products. MCT1 (SLC16A1) is found in the internal BRB's luminous and abluminal membrane [58]. RPE (external BRB) cells express MCT1, MCT3 (SLC16A8), and SMCT1 (SLC5A8) [59, 60]. MCT1 is primarily located in the brush boundary membrane and MCT3 and SMCT1 are located in the outer BRB basolateral membrane. MCT1 can therefore be expected to take monocarboxylic drugs from the circulating blood at the inner BRB and SMCT1 at the outer BRB [61, 62].
- *Nucleoside Drugs:* Adenosine, a purine nucleoside that occurs naturally, has multiple functions in retinal neurotransmission, blood flow, vascular formation, and the response of adenosine receptors to ischemia. Adenosine is conveyed from the blood to the retina by Na+-independent equilibrative nucleoside transporters (ENTs) located at the blood-retina barrier (BRB) [63]. Numerous antiviral and anti-cancer nucleoside medications, including gemcitabine, cytarabine, and zalcitabine, are carried by ENT1 (SLC29A1) and ENT2 (SLC29A2). Moreover, 3'-azide-3'-deoxythymidine (zidovudine, AZT) is present in ENT2. Adenosine and thymidine block the transportation of [3H] adenosine (RUI) from blood to the retina, while cytidine does not affect this process [64, 65].
- *Folate Analog Drugs:* Methotrexate is a drug that is antimetabolite and antifolate and acts by inhibiting folic acid metabolism. It is used as an anticancer drug in the treatment of intraocular lymphoma [66, 67]. Folates are cofactors that help certain amino acids, pyrimidines, and purines be synthesised. They also help homocysteine be converted to methionine. Nutritional amblyopia and retinal toxicity have been linked to deficiencies in retinal folate. Several transporters are involved in the absorption of folate and its analogs, including the decreased folate carrier (RFC1/SLC19A1), folate receptor a, and the H+-coupled folate carrier (PCFT/SLC46A1), including methotrexate. Much of the folate in most mammals' plasma is methyltetrahydrofolate (MTF) in the reduced form.
- *Organic Cationic Drugs:* The BRB functionally expresses organic cation transporters (OCTs and OCTNs) and a transport process (choline transport). Retinal endothelial capillary cells express OCTN2 (SLC22A5) and OCT3 (SLC22A3) RPE cells [68]. OCTN2 mediates the transportation of acetyl-L-carnitine in the inner BRB from the blood to the retina [69]. Although OCTN2 carries verapamil with a number of additional cationic and zwitterionic drugs, such as β-lactam antibiotics as substrates (cephaloridine, tetraethylammonium, pyrilamine, quinidine, and valproate), OCTN2 might not play a significant role in the transfer of verapamil from blood to the retina [70].

Retina-To-Blood Efflux Transport of Drugs

- *ABC Transporters:* The retina's capacity to see systemically accessible substances is limited by efflux transport at the BRB. Therefore, the substrate concentration in retinal interstitial fluid is primarily controlled by a specific efflux transport mechanism. Xenobiotics, such as pharmaceuticals obtained from the bloodstream, are transported out of the inner and outer BRB via ATP-binding cassette (ABC) transporters. This process is seen in **Figure 7**, where the xenobiotics are expelled from both the luminal and basolateral membranes. P-gp is an ATP-dependent transporter that expeditiously eliminates a broad spectrum of chemotherapeutic drugs and lipophilic substances. P-gp, an efflux transporter, is produced by the MDR1 gene in humans and the MDR 1A and MDR 1B genes in rodents. It is primarily located in the luminal membrane of the inner (BRB) and mostly in the basolateral membrane of the outer (BRB) [71, 72]. While minimal levels of cyclosporin A have been found in plasma, there has been no detection of cyclosporin A, a substance that is transported by P-gp, in the intraocular tissues of rabbits, rats, or humans who have been treated with cyclosporin A [73-75]. This study demonstrates the crucial function of P-gp at the BRB in protecting the retina from harmful substances and inhibiting the transportation of drugs to the retina. Apart from P-gp, the internal and external BRB express a number of proteins linked to multidrug resistance (MRP / ABCC) and proteins linked to breast cancer (BCRP / MXR / ABCP / ABCG2) [76].

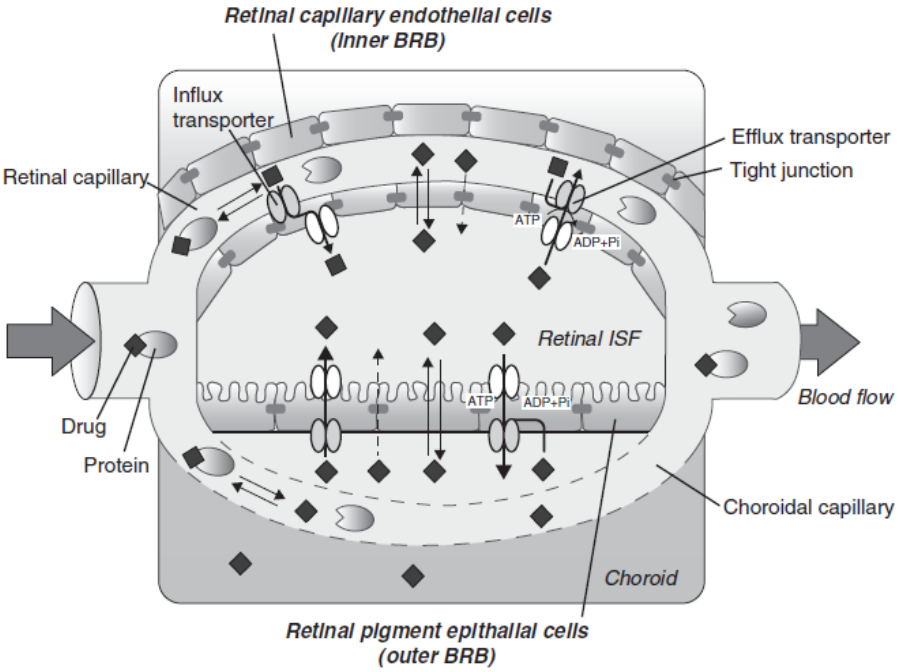


Figure 7. Drug flux through the blood-retinal barrier

MRPs are involved in the transport of negatively charged molecules, such as compounds that are conjugated with glucuronic acid and glutathione. ABCG2 has a preference for both pharmaceuticals (such as mitoxantrone and doxorubicin) and photosensitive toxins, including porphyrin-related pheophorbide, which is a dietary phototoxin generated from chlorophyll. The levels of MRPs transcripts at the internal BRB are measured in isolated mouse and rat retinal capillary endothelial cells [25]. MRP4 (ABCC4) exhibits the greatest amount of transcript, whereas MRP3 (ABCC3) in mice and MRP6 (ABCC6) in mice and rats are expressed at comparatively lower levels [77]. Based on the transcript data, it has been shown that the MRP4 protein is found on the luminal membrane of the internal BRB in mice. Among the six genes that code for MRPs, MRP1, MRP4, and MRP5 are expressed in human RPE cells [78, 79]. Functional investigations indicate that MRPs are present on the basolateral cell membrane of the RPE; however, the precise localization of these MRPs remains unidentified. Rats' internal BRB luminal membrane is home to ABCG2, which may not be expressed in RPE cells [80, 81]. Organic anions are transported from the retina to the blood through two stages: first, they are absorbed by transporters on the brush-border and abluminal membranes, and then they are effluxed into the bloodstream by MRPs and ABCG2 on the luminal and basolateral membranes of the internal and external BRB. The OATP, SLCO, and SLC21A, as well as the OAT and SLC22A families, play a crucial role in the uptake of organic anions from the transporters located on the abluminal and brush-border membranes of the inner and outer BRB.

- *Organic Anionic Drugs:* Treatment of bacterial endophthalmitis with β-lactam antibiotics is less successful due to restricted distribution in the vitreous humor/retina after systemic injection [82]. A number of β-lactam antibiotics, including benzylpenicillin (PCG), are substrates of the organic anion transporter (OAT)3 (SLC22A8) [83]. 6-Mercaptopurine (6-MP) is often used as a chemotherapeutic agent for juvenile acute lymphoblastic leukemia. The frequency of relapse of acute lymphoblastic leukemia affecting the eye in infancy is rare and poses a challenging situation [84]. The narrow range of 6-MP in the eye is likely responsible for this. Digoxin, a selective inhibitor of organic anion transporting polypeptide (OATP) 1A4 (SLCO1A4/OATP2) [85], did not decrease the transport of [3H] PAH, [3H] PCG, and [14C] 6-MP in the retina. However, probenecid, PAH, and PCG, which are relatively specific substrates of OAT3, did lower the transfer. OAT3 is located on the outer side of the membrane of endothelial cells in the capillaries of the retina. PAH, 6-MP, and β-lactam antibiotics are known to be substrates for MRP4. The limited distribution of drugs in the retina and eye may be attributed to the transportation of anionic medications from the retina to the blood across the blood-retina barrier (BRB). Hence, OAT3 and MRP4 play a crucial role in facilitating the absorption of PAH, PCG, and 6-MP in the interstitial fluid, as well as their excretion across the luminal and abluminal membranes of retinal capillary endothelial cells. OAT3 and MRP4 enhance the movement of PAH, PCG, and 6-MP from the vitreous humor and retina into the plasma. This happens via the internal BRB. [3H] After being injected directly into the vitreous humor of rats, estradiol 17-β glucuronide (E17bG) and [3H] dehydroepiandrosterone sulfate (DHEAS), which are conjugates of glucuronic acid and sulfate for neuroactive steroids, are removed from the vitreous humor by a bi-exponential process [86, 87].

  Digoxin, a specific oatp1a4 substratum, and other organic anions significantly reduced the continuous removal level of [3H] E17bG and [3H] DHEAS at the terminal phase, which was double that of D-mannitol. DHEAS efflux was also

blocked in the presence of PAH, an OAT3 substrate that is rather selective. Oatp1a4 is expressed in both RPE cells and rat retinal capillary endothelial cells. Furthermore, capillary endothelium-isolated rat capillary cells express oatp1a4 and 1c1 (Slco1c1/oatp14) mRNA primarily [88].

Oatp1c1 transports E17bG, similar to oatp1a4; however, it does not have a strong affinity for digoxin. MRP4 is recognized as a transporter for the substances E17bG and DHEAS. Oatp1a4 and/or OAT3 transport E17bG and DHEAS from the outer and inner membranes of retinal endothelial capillary cells and RPE cells, and they are expelled from the cells into the bloodstream, probably via MRP4. Since most medically relevant medications are organic anions, they are continuously extracted from the retina throughout the BRB, preventing the accumulation of these drugs at a level that would be therapeutically effective. This includes vaccines, anti-cancer, anti-HIV, and anti-inflammatory agents [89].

However, this hurdle could be overcome if specific organic anion transporter inhibitors are administered together with the drugs. Sunkara *et al*. [90] showed that administering probenecid, a blocker of organic anion transfer, and prior to treatment increases the concentration of N-4-benzoylaminophenylsulfonylglycine retinal, a novel inhibitor of anionic aldose reductase. Inhibiting drug efflux transporters is likely to enhance the distribution of the treatment to the retina by reducing its transit from the retina to the circulation. However, since peripheral tissues and the blood-brain barrier also include efflux transporters, this approach must take into consideration differences in the way that drugs are distributed in these organs [91].

Conclusion

The eye is a complex organ with many hurdles to absorb the drug molecules. Researchers are now exploring many technologies, including implants, carrier systems, targeted particles, viral vectors, and sonotherapy, for overcoming the barriers inside the eye. Retinal diseases including molecular degeneration, diabetic eye disease, and glaucoma affect vision. The BRB is made up of several transporters and close junctions that precisely limit the flow of hydrophilic material from the blood to the retina. ABC transporters like P-gp, ABCG2, and MRP4 are found in the luminal and/or basolateral membranes of the BRB. These transporters act as a structural barrier and restrict the delivery of several lipophilic and anionic drugs. In order to create effective methods for delivering drugs to the retina, specific injection and transport mechanisms inside the BRB might be used.

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