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Review

Potential of Lipoprotein-Based Nanoparticulate Formulations for the Treatment of Eye Diseases

Ryosuke Fukuda\textsuperscript{a,b} and Tatsuya Murakami\textsuperscript{c,d}

\textsuperscript{a}Department of Biotechnology, Graduate School of Engineering, Toyama Prefectural University; \textsuperscript{b}Kurokawa, Imizu, Toyama 939–0398, Japan; \textsuperscript{c}Research Fellow of Japan Society for the Promotion of Science (JSPS); \textsuperscript{d}Kojimachi Business Center Building, 5–3–1 Kojimachi, Chiyoda-ku, Tokyo 102–0083, Japan; \textsuperscript{e}Department of Pharmaceutical Engineering, Faculty of Engineering, Toyama Prefectural University; \textsuperscript{f}5180 Kurokawa, Imizu, Toyama 939–0398, Japan; and \textsuperscript{g}Institute for Integrated Cell-Material Sciences (iCeMS), Kyoto University Institute for Advanced Study (KUIAS); Sakyo-ku, Kyoto 606–8501, Japan.

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Lipoproteins are naturally occurring nanoparticles and their main physiological function is the promotion of lipid metabolism. They can be prepared in vitro for use as drug carriers, and these reconstituted lipoproteins show similar biological activity to their natural counterparts. Some lipoproteins can cross the blood-retinal barrier and are involved in intraocular lipid metabolism. Drug-loaded lipoproteins can be delivered to the retina for the treatment of posterior eye diseases. In this review, we have discussed the therapeutic applications of lipoproteins for eye diseases and introduced the emerging animal models used for the evaluation of their therapeutic effects.

Key words lipoprotein; nanoparticle; lipid metabolism; eye disease treatment; drug delivery system

1. INTRODUCTION

Lipoproteins are nanoparticles that are synthesized and metabolized in the body and help to maintain lipid and cholesterol homeostasis. The history of lipoprotein research dates from the 1930s.\textsuperscript{3} Ultracentrifugal analysis of serum proteins was performed and it was proposed that there were lipoprotein subclasses, known as “spectrum lipoproteins”\textsuperscript{25}. At that time, a lipid–protein complex was designated as “Protein X.” Subsequently, $\alpha$- and $\beta$-lipoproteins were identified by electrophoresis and chemical plasma protein fractionation.\textsuperscript{3,4} Later, an association between a series of lipoproteins and the risk of cardiovascular diseases was reported,\textsuperscript{5,6} and the new lipoprotein subclasses indicated below were established.\textsuperscript{7}

As the human population is increasing and aging, the number of people affected by blindness arising from eye disease has significantly increased.\textsuperscript{8,9} Vision loss significantly reduces QOL. As most of the causes of blindness are disorders of the posterior eye segment, the delivery of the drug to the posterior eye segment is required. Although there are effective treatments for eye diseases, many are invasive. Various nanoparticles and antibodies have been used as treatments, most of which have been injected intravitreally\textsuperscript{10–12} or subconjunctivally.\textsuperscript{13} Thus, the development of safer and more diverse treatments is desired. The main reason that the method of drug delivery to the posterior segment of the eye is not widespread is that the eye has multiple barrier functions, such as the corneal layer structure and the blood–retinal barrier (BRB).

However, it was previously shown that several circulating lipoproteins translocate into the retina via lipoprotein receptors expressed on retinal cells.\textsuperscript{14,15} Another study showed that major transporters and molecules associated with lipoprotein metabolism are expressed in human and monkey retina.\textsuperscript{15} These observations suggested that some lipoproteins are taken into the retina via receptors on retinal cells and that there are some systems of lipid metabolism in the retina. Thus, a strategy for drug delivery to the retina using lipoproteins would be rational. In this review, we first introduce the biochemical aspects of lipoproteins and then summarize the recent findings of the relationship between lipoproteins and eye diseases, as well as their potential for the development of ocular drug delivery systems.

2. BIOCHEMICAL PROPERTIES AND LIPOPROTEIN BIOSYNTHESIS

Lipoproteins are natural nanoparticles containing lipids and lipid-binding proteins, apolipoproteins (apos).\textsuperscript{16–20} Plasma lipoproteins are classified into five major groups based on their density: chylomicrons (CMs), very low-density lipoproteins (VLDLs), intermediate-density lipoproteins (IDLs), low-density lipoproteins (LDLs), and high-density lipoproteins (HDLs).\textsuperscript{21} The biochemical properties of lipoproteins are summarized in Table 1. These lipoproteins have the capability to transfer cholesterol in the bodies.\textsuperscript{22}

The biogenesis of lipoproteins is a complex series of processes (Fig. 1). HDL generation starts from the secretion of the lipid-free or lipid-poor apoA-I by the liver and the intestine\textsuperscript{22–25} and then phospholipids and cholesterol are transferred to apoA-I by the interaction of apoA-I and the
ATP-binding cassette transporter A1 (ABCA1). The apoA-I/phospholipid/cholesterol complexes are gradually converted to discoidal particles (nascent HDL). Discoidal HDL is converted to the spherical form (mature HDL) by the esterification of cholesterol by lecithin-cholesterol acyltransferase (LCAT).26) Mature HDL is taken up by the liver via scavenger receptor class B type I (SR-BI).27) Then, mature HDL transfers a part of cholesteryl esters to LDL by cholesteryl ester transfer protein (CETP), while receiving triglycerides (TG) from LDL to form a TG-rich HDL.28) Other lipoproteins are produced in the process of lipoprotein metabolism, as mentioned below.

Lipids in dietary fats are absorbed from the small intestine and become CM, which is transported into the blood.29,30) After neutral fats in CM are depleted by lipoprotein lipase (LPL), they become smaller and cholesterol-rich CM remnants; subsequently, they are taken into the liver.31–33) In the liver, cholesterols, neutral fats, and apoB-100 are synthesized, and VLDL is formed with them and then secreted into the blood.34–36) VLDL is metabolized by LPL to IDL and further metabolized to LDL by the action of hepatic lipase.37,38) LDL is taken up by LDL receptors and supplies cholesterols to the peripheral tissues.20,39,40) LDL that has completed its role is taken up and collected by the liver.39)

### 3. PHYSIOLOGICAL FUNCTIONS OF LIPOPROTEINS

The physiological functions of lipoproteins are diverse and related to their biosynthesis. As described later, defects in some of these functions are associated with cardiovascular diseases.41) CM is synthesized by the intestine and is responsible for the transport of dietary triglycerides and cholesterol from the intestinal epithelium to cells in the body. VLDL is synthesized in the liver and is responsible for the delivery of triglycerides and cholesterol from the liver to various tissues. Triglycerides of VLDL are hydrolyzed in the plasma by LPL, and converted into IDL. As triglycerides and cholesterol are further delivered to various tissues, IDL will gradually become smaller and will be converted into LDL, the final product of VLDL catabolism. LDL is the major cholesterol-carrying lipoproteins in the plasma and elevated LDL levels in the plasma or defective LDL receptors are associated with an increased risk of cardiovascular disease. In early stages of atherosclerosis, LDL accumulates in the extracellular subendothelial space of arteries, and then is subjected to oxidation mediated by lipoxygenase, myeloperoxidase, reduced nicotinamide adenine dinucleotide phosphate (NADPH) oxidase, and nitric oxide synthase.42–46) Oxidized LDL contributes to the expression of pro-inflammatory cytokines, chemokines, and
HDL is responsible for the transport of cholesterol from cells back to the liver and exerts a protective effect against atherosclerosis. It acts as an antioxidant agent, protecting against the generation of oxidized lipids by the action of redox-active Met residues of apoA-I or receiving lipid hydroperoxides from oxidized LDL via SR-BI. The oxidized HDL tended to be more rapidly and selectively removed by Hep G2 cells than native HDL. Dysfunctional HDL reduces the capacity to inhibit the expression of adhesion molecules and exerts impaired antioxidative activity.

HDL shows anti-inflammatory effects as well as antioxidative effects. For the suppression of inflammation, statin-loaded HDL is used, whereas HDL itself acts as an effective inflammation suppressor. Anti-inflammatory activity is thought to occur via receptor-mediated reaction. The binding of HDL to SR-BI induces cholesterol efflux and the subsequent suppression of the activation of the transcription factor nuclear factor-kappaB (NF-κB). Thus, HDL is thought to be a negative risk factor for the development of atherosclerosis.

4. RECONSTITUTION OF LIPOPROTEINS

Wild type or mutant apoA-I, apoB-100, and apoE can be obtained by genetic modification technology using Escherichia coli or transgenic mice expression systems. apoA-I has a tandem repeat of 10 amphiphilic α-helices. In particular, various deletion mutants of apoA-I, such as N-terminal and/or C-terminal deletion mutants, are well studied. By using the recombinant apoA-I expression system, HDL can be reconstituted in vitro without the extraction of native apoA-I from human plasma. It was suggested that the N-terminal region of apoA-I residues 1–41 may not have significant effects on the ABCA1-dependent cholesterol and phospholipid efflux ability, and that residues 220–231 were necessary for the interaction between apoA-I and ABCA1 to generate spherical HDL in mice, indicating that the C-terminal domain was essential for the ABCA1-dependent spherical HDL (but not discoidal HDL) formation in vivo. For apoB-100 and apoE, LDL receptor-binding domains have been reported to be localized to the residues 3147–3157, 3359–3367, and 3345–3381, etc., for apoB-100, and 140–150 for apoE, but it is still unclear whether the LDL receptor-binding domains of apoB-100 interact with the LDL receptor independently or cooperatively.

There are several approaches for the synthesis of HDL mimics without naturally derived apoA-I. HDL mimics are reconstituted from recombinant apoA-I or their mimetic peptides and phospholipids. Representative apoA-I mimetic peptides are the 18A peptide and the 4F peptide. These synthetic peptides show similar physiological actions, as apoA-I. 18A peptide stimulates as much cholesterol efflux as apoA-I, and 4F peptide shows anti-atherosclerotic effects. In addition, HDL mimics containing two tandem repeats of the 4F peptide linked with an apoA-I interhelical sequence showed higher ABCA1-mediated cholesterol efflux than full-length mouse apoA-I. These peptides do not have any similarity to the apoA-I amino acid sequence, but all have amphiphilic helical structures that show lipid-binding affinity. This implied that the apoA-I amino acid sequence was not necessary for HDL reconstitution and its physiological action. By using apoA-I mimetic peptides, new strategies for the enhancement of its physiological activity have been investigated. A recent study reported that the oligomerization of the apoA-I mimetic peptide enhanced physiological function (stability against enzymatic digestion, plasma half-life, and pharmacokinetic profile) of HDL mimics compared with HDL mimics consisting of monomeric apoA-I mimetic peptide. The reconstitution of LDL and CM has also been studied with recombinant apolipoproteins, lipids, cholesterol, and cholesteryl esters. By making use of the knowledge on LDL receptor-binding domains, synthetic LDL particles capable of promoting the proliferation of U937 cells were prepared. CM mimics containing human recombinant apoE for protection against endotoxemia were also synthesized.

From a biochemical perspective, the reconstitution of HDL and LDL from its main components (lipid and apoA-I/apoB-100) has been explored. Several reconstitution methods have been proposed: cholate dialysis, liposome hydration, and more recently, microfluidics. LDL has been prepared by cholate dialysis and microemulsion/apoB-100 reassembly methods. The procedures involved in cholate dialysis or liposome hydration are divided into three main steps, lipid nanoparticles formation, the addition of apolipoproteins, and purification, which are labor-intensive processes requiring at least a few days. These methods have been utilized mainly for laboratory-scale HDL/LDL reconstitution. In contrast, the microfluidic approach was developed as a continuous and large-scale HDL reconstitution method with high reproducibility, yield, and homogeneity. These methods enabled us to prepare a variety of HDL-like nanoparticles containing apoA-I mutants or apoA-I mimetic peptides.

5. DRUG INCORPORATION INTO LIPOPROTEINS

Many studies have demonstrated that hydrophobic drugs can be incorporated into lipoproteins and these lipoproteins can act as drug carriers. CM-mimicking nanoemulsions have drug-loading potential and drug-release properties, which can be attributed to drug delivery via the lymphatic pathway. A large amount of coumarin-6, doxorubicin hydrochloride, or IR780, was loaded into native LDL (10 ± 1, 25 ± 3, 73 ± 4 drugs per particle, respectively), and used to facilitate in vivo imaging. Discoidal reconstituted HDL, termed nanodisks, are also potential drug carriers into which a variety of drugs can be loaded, such as anticancer drugs and small interfering RNA (siRNA)-cholesterol conjugates and imaging agents. We successfully observed that the therapeutic benefit of doxorubicin was improved in tumor-bearing mice by complexation with a nanodisk genetically modified with a cell-penetrating peptide. Our previous study also demonstrated that drug-loading efficiency could be controlled by temperature. Most of the cargos incorporated into lipoproteins are hydrophobic, and it appears to be difficult to incorporate hydrophilic molecules into the lipid membrane of lipoproteins, unless membrane-anchoring molecules, such as cholesterol or fatty acids, are conjugated to them. In contrast, recent studies showed that hydrophobic ion pairing could improve drug encapsulation into lipid nanocarriers. This technique may enable the incorporation of hydrophobic mol-
The eye consists of many tissues and it has photoreceptor cells of the neural retina and transmitting them to the brain. The eye consists of many tissues and it has photoreceptor cells of the neural retina and transmitting them to the brain. The retina also has a complex structure, consisting of pigment epithelium, Müller cells, horizontal cells, bipolar cells, amacrine cells, ganglion cells, and others.  

6. PRESERVATION OF LIPOPROTEINS

Freeze-drying in the presence of sucrose is a fundamental strategy for the preservation of pharmaceutical formulations. Although amounts of reconstituted HDL in aqueous solutions changed after storage 30°C for a few days, those freeze-dried in the presence of sucrose were stable at 30°C for 2 years. In contrast, pre-β-lipoproteins (VLDL) were found to degrade through the freeze-drying process, although α-lipoproteins (HDL) and β-lipoproteins (LDL) were not. Freeze-drying is therefore useful for the practical development of reconstituted HDL formulations.

7. STRUCTURE AND FUNCTION OF THE EYE

The eye is a small organ delivering light signals to the photoreceptor cells of the neural retina and transmitting them to the brain. The eye consists of many tissues and it has a complex structure (Fig. 2). The eye globe is essentially a sphere consisting of three layers. The outermost layer is the cornea and the sclera. The cornea is the transparent and connective tissue that covers the front of the eye, and directs the light to the retina. The sclera forms a smooth connective tissue that is mainly composed of collagen and elastic fibers, and has a role in maintaining the shape of the eye. The middle layer is the vascular tunic or uvea, consisting of the iris and the ciliary body in the anterior segment, and the choroid in the posterior segment of the eye. The iris is a thin film located between the cornea and the lens. It plays a role in adjusting the amount of light entering the retina, by changing the size of the pupil. The ciliary body is close to the iris, and is the source of production the aqueous humor. The choroid extends from the edge of the optic nerve to the ciliary body. It is responsible for ocular nutrition through the systemic blood supply. Finally, the innermost layer consists mainly of the neural retina. The retina is the most important tissue because it captures images and transmits the signals to the brain.

8. OCULAR BARRIERS TO DRUG ENTRANCE

In the ocular system, there are several barriers in the cornea and at the blood-ocular interface. The cornea consists of five components: corneal epithelial cells, Bowman’s membrane, the corneal stroma, Descemet’s membrane, and corneal endothelial cells. The cornea acts as the conventional site of drug entry into the eye, especially for eye drops. However, the cornea serves as a major barrier to the absorption of eye drops. The cornea provides both a physical barrier and a metabolic barrier. The structure of the cornea is similar to a lipid-water-lipid “sandwich,” functioning as both a hydrophilic barrier and a hydrophobic barrier. In addition, the zonula occludens, or tight junctions between the corneal epithelial cells further limit the drug penetration.

The sclera protects the eye from external force and injury, and acts as a barrier to entrance into the eye for various substances. The diffusion of drugs inside the sclera is known to depend on several factors, including the molecular weight and the degree of polarity. Previous studies have found that human sclera is permeable to hydrophilic compounds up to approximately 150 kDa in molecular weight.

There are important two blood-ocular barriers in the eye. The first is the blood-aqueous barrier (BAB), in which the blood and the aqueous humor freely exchange. The BAB helps the metabolism in the cornea and lens and the exchange between blood and the intraocular fluids. The second is the BRB, which helps to regulate the flow in the posterior segment of the eye and is important in the development of the vascular retinopathies and retinal edema.

9. LIPID METABOLISM AND EYE DISEASES

There are many causes of blindness, including cataract, age-related macular degeneration (AMD), glaucoma, and diabetic retinopathy; most are diseases of the posterior segment of the eye. Lipid metabolism plays an important role in the prevention of vision loss. It was suggested that the delivery of serum cholesterol to the retinal pigment epithelium (RPE) and also possibly Müller cells was mediated mainly by LDL in rats (Fig. 3). In addition, the major transporters and molecules associated with lipoprotein metabolism are expressed in the retina. ABCA1 and apoA-I are localized to the ganglion cell layer, RPE, and rod photoreceptor inner segments. LCAT and CETP are localized mainly to the interphotoreceptor matrix. SOR-BI is localized mainly to the ganglion cell layer and photoreceptor outer segments. Recent reports have demonstrated that cholesterol transport within the retina is mainly mediated by locally synthesized HDL and LDL, with a clear association between plasma and retinal lipid levels.

Abnormalities or defects in these molecules related to lipid metabolism has deleterious effects on field of view. For example, familial LCAT deficiency is associated with the occurrence of the fish-eye disease, which increases corneal opacity. Fish-eye disease is characterized by the inability to esterify cholesterol in HDL, which leads to an increase in free cholesterol in the plasma. In addition, several studies indicated that the lack of antioxidant (e.g., vitamin E) or lipid peroxidation can cause morphological alteration in the retina. Lipid peroxide accumulation occurs in retinal
degeneration and lipid peroxide formation is thought to be associated with functional changes in the retina. Accordingly, if lipid peroxides in LDL are not transferred to HDL, retinal disorders will be caused by the breakdown of the lipid transfer mechanism mentioned above.

It is known that patients with diabetes may be at risk of glaucoma. Glaucoma is a neurodegenerative disorder of the optic nerve that causes vision loss. Diabetes and glaucoma have a common pathophysiologic mechanism: lipid metabolism abnormalities, which cause oxidative stress and promote cellular apoptosis. The occurrence of diabetes increases triglyceride secretion, whereas the clearance of lipids and lipoproteins decrease. As a result, inflammation and oxidative stress increase, leading to neuronal dysfunction.

AMD is another ocular disease leading to severe vision loss, and its pathogenesis involves dyslipidemia. In all stages of AMD, patients have high concentrations of ‘extra-large’ HDL, which was thought to be a driver of AMD. In contrast, another group reported that patients in the late stages of wet AMD, in which abnormal blood vessels grow in the macula, had high concentrations of medium-sized (8.2–8.8nm) HDL and IDL and low concentrations of CM and VLDL, whereas patients with early AMD did not. Furthermore, a missense mutation in the CETP gene, which is seen in approximately 6% of individuals of East Asian descent, increased the risk of AMD by 70%. However, a missense mutation in the CETP gene did not significantly alter the association between the plasma HDL concentration and the increased risk of wet AMD. Thus, the authors deduced that the effect of the defect in the CETP gene on wet AMD risk may not be mediated through HDL in systemic circulation, but by local events in the retina. This implied that a CETP gene mutation inhibited HDL maturation locally in the retina via the inhibition of cholesteryl ester transfer between lipoproteins, resulting in wet AMD. A similar situation is observed in cardiovascular diseases, in which the quality of HDL (cholesterol efflux capacity of HDL), rather than the quantity of cholesterol in HDL (plasma HDL-cholesterol concentration), was a better predictor. On the basis of these reports, the relationship between the blood lipoprotein levels and the pathogenesis of AMD remains to be established, whereas local intervention to control the level in the posterior segment of the eye would be helpful.

AMD pathogenesis is strongly related to inflammation and many inflammatory molecules, such as soluble intercellular adhesion molecule-1 and tumor necrosis factor-α receptor II, are proposed as biomarkers of AMD. Choroidal neovascularization (CNV) in the posterior macula causes wet AMD. In addition, the transplantation of microglia producing various pro-inflammatory cytokines, such as tumor necrosis factor-α (M1 microglia), into the subretinal area promotes CNV, which supports the involvement of inflammation in AMD. In the early stages of AMD, patients are at risk of developing other systemic diseases, such as coronary heart disease. Thus, preventive measures against AMD are important for preserving health.

10. POTENTIAL OF LIPID OR LIPOPROTEIN-BASED NANOPIRTE FORMULATIONS FOR OCULAR DRUG DELIVERY SYSTEMS

Lipoproteins are utilized for the treatment of posterior eye diseases. From the discovery of lipoproteins able to serve as carriers of benzoporphyrin derivatives, lipoprotein-based nanoparticles containing benzoporphyrin derivatives have been developed for photodynamic therapy of choroidal neovascularization. Visudyne, a well-known liposomal formulation containing verteporphyrin, a benzoporphyrin monoacid. The mechanism of the delivery of this compound to the posterior eye is thought to be the rapid transfer of benzoporphyrin derivatives to plasma LDL after intravenous
injection, followed by the accumulation into neovascular endothelial cells via LDL receptors.\textsuperscript{139} However, as RPE cells also express LDL receptors,\textsuperscript{140} damage to RPE cells may be inevitable.\textsuperscript{136}

Eye drops are an excellent candidate for daily eye care practice. However, the development of eye drops for the preventive treatment of wet AMD are challenging due to the low drug delivery efficiency to the posterior segment of the eyes, which is caused by the ocular barriers as described above. Therefore, some strategies are necessary for the penetration of drugs into the cornea. Hironaka reported that small (approx. 100 nm) liposomes could deliver cargo (coumarin 6) to the posterior segment of the eye and that the delivery efficiency was enhanced by smaller liposomes, saturated phospholipids, and a cationic polymer on the surface, which all increased the residence time on the corneal surface and improved stability.\textsuperscript{140,141}

The current treatment of AMD is performed through the intravitreal administration of anti-angiogenic proteins ranibizumab or bevacizumab,\textsuperscript{142,143} or the intravenous injection of liposomal verteporfin followed by laser illumination.\textsuperscript{144} There are no clinically approved eye drops for the treatment of wet AMD.\textsuperscript{145} This HDL mutant consists of phospholipids with two stearic acids, which form a relatively rigid membrane, and apoA-I genetically fused with a cell-penetrating peptide (penetratin) to its C-terminus. Furthermore, HDL is classified as one of the smallest nanocarriers (approx. 10nm).\textsuperscript{146} These properties of amphiphilicity, cell-penetrating ability, and small size are advantageous for the penetration of the cargo into the cornea. Indeed, pazopanib-loaded HDL mutant eye drops successfully suppressed CNV in mice, indicating that the drug delivery to the posterior eye segment was achieved with the HDL mutant to some extent (Fig. 4). In this study, the HDL mutant was thought to be delivered to the posterior retina within 10 min. For the drug delivery route to the posterior eye segment, two possible pathways were discussed: corneal-vitreal and conjunctival-scleral. In addition, this HDL mutant itself provided a therapeutic benefit in this model system, which would be derived from the physiological functions of HDL. As mentioned above, the use of lipoproteins in ocular drug delivery systems is growing, especially for the treatment of AMD, and various strategies to control their functions will be developed owing to the similarity between the pathogenesis of AMD and atherosclerosis.

11. HDL AND OTHER DISEASES

Drug delivery to the brain is extremely difficult because...
the transfer of substances from the blood to brain tissue is strictly limited by the blood–brain barrier (BBB). The brain capillary endothelial cells have tight junctions between them, which limit the transfer of substances. Therefore, even if most drugs are administered into the blood, they are not transferred to the brain tissue. In contrast, the LDL receptor is known to be expressed at the BBB and biochemical studies have demonstrated that LDL was specifically transcytosed across a BBB-mimicking cultured cell monolayer, which has formed a basis for nanoparticulate drug delivery to the brain. After reporting the feasibility of penetration into the BBB, it has been found that apoE and possibly apoB promote the transport of drugs bound to nanoparticles across the BBB. Briefly, intravenously injected apoE- or apoB-coated polystyrene 80 nanoparticles or apoE-conjugated albumin nanoparticles are delivered to the brain.  

The transfer mechanism of the uptake of apoE-conjugated albumin nanoparticles into the cerebral endothelium was reported to be transcytosed to the brain parenchyma via endocytosis. Owing to these discoveries, LDL receptors are consider as targets of brain drug delivery and a recent study demonstrated the penetration of HDL mimics consisting of apoE3 and a phospholipid bilayer (ApoE3-rHDL) through the BBB. In addition, ApoE3-rHDL showed high binding affinity to amyloid β, which is believed to accumulate in the brain of patients with Alzheimer’s disease, and decreased amyloid β deposition.

Lipoproteins are useful cancer therapy agents as they have the capacity to carry many hydrophobic anticancer drugs, as mentioned before. Their structure confers inherent drug-carrying ability; of the lipoproteins, HDL is the smallest, with a diameter of approx. 10 nm. These characteristics of HDL allow us to predict their passive targeting to tumor tissues based on the enhanced permeability and retention effect. Tumor tissues have significantly higher vascular permeability than normal tissues, and their lymphatic system is not developed well, which allows biocompatible macromolecules and nanoparticles to extravasate from tumor blood vessels and be retained there. In this context, it has been reported that HDL mimics were targeted to tumor tissues in mice. More targeted cancer therapies are expected in combination with photothermal and photodynamic therapies.

12. NEW IN VIVO MODELS OF EYE DISEASES

For the therapeutic effects evaluation of newly developed nanoparticulate formulations, rodents and rabbits are mainly used as model animals. In contrast, animal models with higher reproducibility and lower experimental costs and labor requirements are desired. Recently, zebrafish (Danio rerio) has attracted attention as a novel experimental model for in vivo studies of nanoparticulate formulations. Zebrafish are classified as a nonmammalian vertebrate; approximately 70% of protein-coding human genes are related to the zebrafish genes and 82% of genes known to be associated with human diseases have a zebrafish counterpart. Moreover, zebrafish embryos have the unique advantage of optical transparency. Conventionally, zebrafish have been used for studying organogenesis by using morpholino antisense oligonucleotides to silence gene expression and genome editing using TALEN or the CRISPR-Cas system. Using one of these methods, the pharmacokinetics and biodistribution studies of nanoparticulate formulations using transgenic zebrafish expressing fluorescent proteins in the vasculature or macrophages have been reported. The pharmacokinetic data from zebrafish are correlated with data from rodents, indicating that zebrafish can be used as a screening tool for nanoparticulate formulations.

The structure and development of zebrafish ocular tissues have also been studied. The zebrafish cornea consists of relatively undifferentiated cells up to 60 h post fertilization, with the cornea observed at 3 d post fertilization. Furthermore, at 6 months post fertilization, zebrafish are reported to have a corneal tissue ultrastructure consisting of five layers, similar to the structure of the human cornea. More recently, it has been demonstrated that CNV can be induced in zebrafish embryos and adults exposed to hypoxic conditions, which enabled the screening of ocular angiogenesis inhibitors. As for adult zebrafish, the in vivo imaging of retinal vasculature is possible by using a commercially available confocal scanning laser ophthalmoscope. Given these properties, zebrafish have attracted attention as a model system for the investigation of the mechanism of eye diseases. Although rodents and rabbits are still more prevalent candidates for animal models, zebrafish are a common animal model for the early-stage screening of nanoparticulate formulations for the treatment of eye diseases.

13. CONCLUSION

In this review, the biochemical and structural properties of lipoproteins and their applications for ocular drug delivery are briefly summarized. The detailed composition and the structure of the eye, especially the posterior segment of the eye, have been explained, and it has been found that some plasma lipoproteins, such as HDL and LDL, can migrate into the retina. As a result, drug delivery systems based on lipoprotein formulations have been developed. Given that the emerging evidence suggests the close involvement of lipoproteins in ocular diseases, and the preparation of lipoprotein mimics, especially those based on HDL, has become easier owing to the use of short peptides and/or microfluidic method, the development of lipoprotein-based drug formulations with improved drug delivery efficiency and therapeutic activity is a logical step expected in the future.

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