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Rabbit Models of Ocular Diseases: New Relevance for Classical Approaches

Evgeni Y. Zernii[1], Viktoriia E. Baksheeva[1], Elena N. Iomdina[2], Olga A. Averina[1], Sergei E. Permyakov[3], Pavel P. Philippov[1], Andrey A. Zamyatnin Jr.[1,4] and Ivan I. Senin[*,1]

[1]*Belozersky Institute of Physico-Chemical Biology, Lomonosov Moscow State University, Moscow, 119992, Russia;* [2]*Moscow Helmholtz Research Institute of Eye Diseases, Moscow, 105062, Russia;* [3]*Institute for Biological Instrumentation of the Russian Academy of Sciences, Pushchino, Moscow Region, 142290, Russia;* [4]*Institute of Molecular Medicine, Sechenov First Moscow State Medical University, Moscow, 119991, Russia*

Ivan I. Senin

Abstract: Over 100 million individuals are affected by irreversible visual impairments and blindness worldwide, while ocular diseases remain a challenging problem despite significant advances in modern ophthalmology. Development of novel drugs and drug delivery mechanisms, as well as advanced ophthalmological techniques requires experimental models including animals, capable of developing ocular diseases with similar etiology and pathology, suitable for future trials of new therapeutic approaches. Although experimental ophthalmology and visual research are traditionally performed on rodent models, these animals are often unsuitable for pre-clinical drug efficacy and safety studies, as well as for testing novel drug delivery approaches, e.g. controlled release of pharmaceuticals using intra-ocular implants. Therefore, rabbit models of ocular diseases are particularly useful in this context, since rabbits can be easily handled, while sharing more common anatomical and biochemical features with humans compared to rodents, including longer life span and larger eye size. This review provides a brief description of clinical, morphological and mechanistic aspects of the most common ocular diseases (dry eye syndrome, glaucoma, age-related macular degeneration, light-induced retinopathies, cataract and uveitis) and summarizes the diversity of current strategies for their experimental modeling in rabbits. Several applications of some of these models in ocular pharmacology and eye care strategies are also discussed.

Keywords: Ocular diseases, experimental animal models, rabbit, ocular pharmacology, dry eye syndrome, glaucoma, age-related macular degeneration, cataract, uveitis.

INTRODUCTION

A wide variety of ocular diseases leads to severe visual impairments and ultimately to blindness. Although some of these diseases are rare, several eye pathologies, such as cataract, age-related macular degeneration (AMD), glaucoma, dry eye syndrome (DES) and uveitis, are very common (Table **1**). Development of effective strategies for treatment of ocular diseases requires detailed understanding of their etiology, pathogenesis and progression, and therefore recapitulation of these aspects in experimental models [1]. Ocular damages in humans are usually revealed during diagnostics, thus hindering studies of their development from the onset of a disease. Additionally, lack of detailed examinations of human retinas obtained at equivalent pathologic states makes it difficult to compare the results and interpret their pathomorphologies. Therefore, obtaining a suitable and relevant animal model is invaluable for characterizing ocular diseases. In recent decades, a great number of studies were focused on understanding of the pathogenesis and improvement of therapy of ocular diseases, stimulating a search for their experimental animal models. Various species including monkeys, horses, pigs, dogs, cats, rabbits and rodents were used to achieve this goal [2-6]. Although each of these models can mimic certain aspects of ocular diseases, none of them are ideal for their complex studying. Evidently, non-human primates are the only animals capable of developing clinical presentation of ocular diseases closely resembling those seen in humans. However, experiments on monkeys require special laboratory facilities, making them inaccessible to the majority of researchers. Animal management and confinement issues are the main cause of a limited use of equine and porcine models, while dogs and cats are difficult to handle due to their aggressive nature, thus restricting their applications in experimental ophthalmology. The main disadvantage of mouse and rat models is their small eyeball, making it hard to access it for clinical manipulations and thus limiting biomedical applications of these animal models of ocular diseases. Additionally, rodents are poorly suitable for testing novel drug delivery approaches, such as controlled release of pharmaceuticals using drug-eluting intraocular lenses or intra-ocular implants [7]. Moreover, these species are not suitable for pre-clinical efficacy and safety studies due to significant differences in pharmacokinetics and required dosages compared to humans [8].

The limitations described above may be partly overcome by using rabbits (*Oryctolagus cuniculus*) as experimental

*Address correspondence to this author at the Belozersky Institute of Physico-Chemical Biology, Lomonosov Moscow State University, 1-40 Leninskye gory, Moscow, 119992, Russia; Tel: +7(495) 939 23 44; Fax: +7(495) 939 09 78; E-mail: senin@belozersky.msu.ru

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Table 1. Leading causes of ocular discomfort and visual loss*

Disease	Description	Risk factors	Number of Patients
Age-related macular degeneration	Development of degenerative lesions in the macular (or central) retina, affecting older people	Aging, genetic predisposition, arterial hypertension, obesity, prolonged light exposure, tobacco use [129, 130, 134-137]	30-50 million, 8,7% of world blindness cases
Cataract	Clouding of the lens of the eye that impedes the passage of light	Aging, trauma, diabetes, genetic predisposition, prolonged exposure to sunlight, tobacco use [214-216]	20 million, 51% of world blindness cases
Dry eye	Altered tear secretion by lacrimal glands and/or tears evaporation associated with damage to the ocular surface	Aging, autoimmune disorders (Sjogren's syndrome), Graves-Basedow`s disease, diabetes, infections (mononucleosis, cytomegalovirus, AIDS), application of certain anesthetics and antiseptics, wearing contact lenses, laser correction surgery [26, 36, 40, 46, 53]	From 5% to 34% of population, depending on the region
Glaucoma	Structural change and functional deficit of the optic nerve, caused by apoptosis of ganglion cells	Aging, genetic predisposition, steroid intake, trauma and intravitreous surgery [90, 91, 100, 101, 108]	45 million (for open-angle glaucoma), 12% of world blindness cases
Uveitis	Eye inflammation affecting the vascular middle layer of tissue in the eye wall (uvea)	Genetic predisposition, infection, trauma, autoimmune or inflammatory disease [236-238]	1 in 4500 people worldwide, 10-20% of world blindness cases

*According to investigation of World Health Organization (http://www.who.int).

models [6-8], while several rabbit varieties, including albino (New Zealand White) and pigmented (Dutch Belt) are primarily used in ophthalmic research. Generally rabbits are easily handled, rapidly breeding and represent one of the most cost-effective species among animal models. Despite their relatively large size, rabbits are classified as small experimental animals, thus requiring only local animal ethics committee approval [9]. Furthermore, rabbits have relatively large eye, sharing many anatomical features with humans, including eyeball size, its internal structure and optical system, biomechanical and biochemical features, as well as conjunctiva cavity volume (Fig. **1**) [10]. Unlike rodents, traditionally used in laboratory research, rabbits are larger and have prolonged life span, thus enabling studies of age-related aspects of eye diseases by using animals of different ages. Their ocular surface is easily accessible, being an important feature for some models. Additionally, rabbit retina's blood vessels are more superficial and, therefore, more accessible for injections [6]. The above-mentioned features make rabbit eyes suitable for various surgical manipulations (cataract removal, intraocular lens (IOL) insertion, corneal transplantation, laser refractive procedures, glaucoma shunt implantation), as well as for intravitreal and subretinal injections [3, 11]. Finally, rabbits share many common traits with humans, including similar physiology and heterogeneous genetic background. Indeed, phylogenetically rabbits are closer to primates than to rodents [12]. All these features are particularly important, making rabbits highly suitable for testing safety and efficacy of novel approaches for treatment of ocular diseases [7, 8].

Although rabbit and human eyes share many similarities, a number of important unique anatomical features should be considered when working with rabbit models (Fig. **1**) [6, 13]. The anterior segment of a rabbit eye is larger and its crystalline lens occupies significant space, resulting in iris bulging and curvature of the anterior chamber. The diameter of a crystalline lens measured by capsular bag in phakic eyes is comparable in both rabbits and humans (9.95±0.24 mm in rabbit and 9.53±0.31 mm in human eyes), but after excision it changes non-uniformly to 10.47±0.31 mm and 9.58±0.27 mm in rabbits and humans, respectively [14]. Rabbits are capable to protractedly resist blinking due to the unique composition of their tears, containing lipids, produced by Hardarian gland, absent in primates [15]. There are some differences in the lamina cribrosa and its vascular supply, prelaminar optic nerve head and a retinal ganglion cell layer [16]. Unlike its human counterpart, the rabbit retina is partially myelinated by oligodendrocytes [17].

Until now, a number of reviews have focused on applications of various laboratory animals in visual research [2, 3, 5, 18-21]. However, there is a lack of a systematic study describing rabbit models of ocular diseases to the best of our knowledge. Although rabbits have been employed in laboratory studies for a long time, recent advances in novel treatment strategies for severe visual impairments open new avenues in using these animal models in experimental ophthalmology [7, 22-25]. The goal of this review is to describe rabbit models of the most common ocular diseases including DES, glaucoma, AMD, light-induced retinopathies, cataract and uveitis. In each case, a brief description of clinical, morphological and mechanistic aspects of a respective disease, and strategies for its experimental modeling in rabbits, as well as biomedical applications of the resulting models are summarized.

DRY EYE SYNDROME

DES, or keratoconjunctivitis sicca, is a common ophthalmological disorder involving approximately 5-34% of word population with increased prevalence upon aging [26]. Being a multifactorial disease, DES is characterized by altered tear secretion by lacrimal glands and/or tears evaporation affecting ocular surface (eye ball, sclera, conjunctival sac and cornea). The general symptoms of DES include discomfort and visual disturbance associated with increased osmolarity and instability of tear film and injuries in the ocular surface caused by subacute inflammation [27]. DES patients possess significantly reduced reading speed

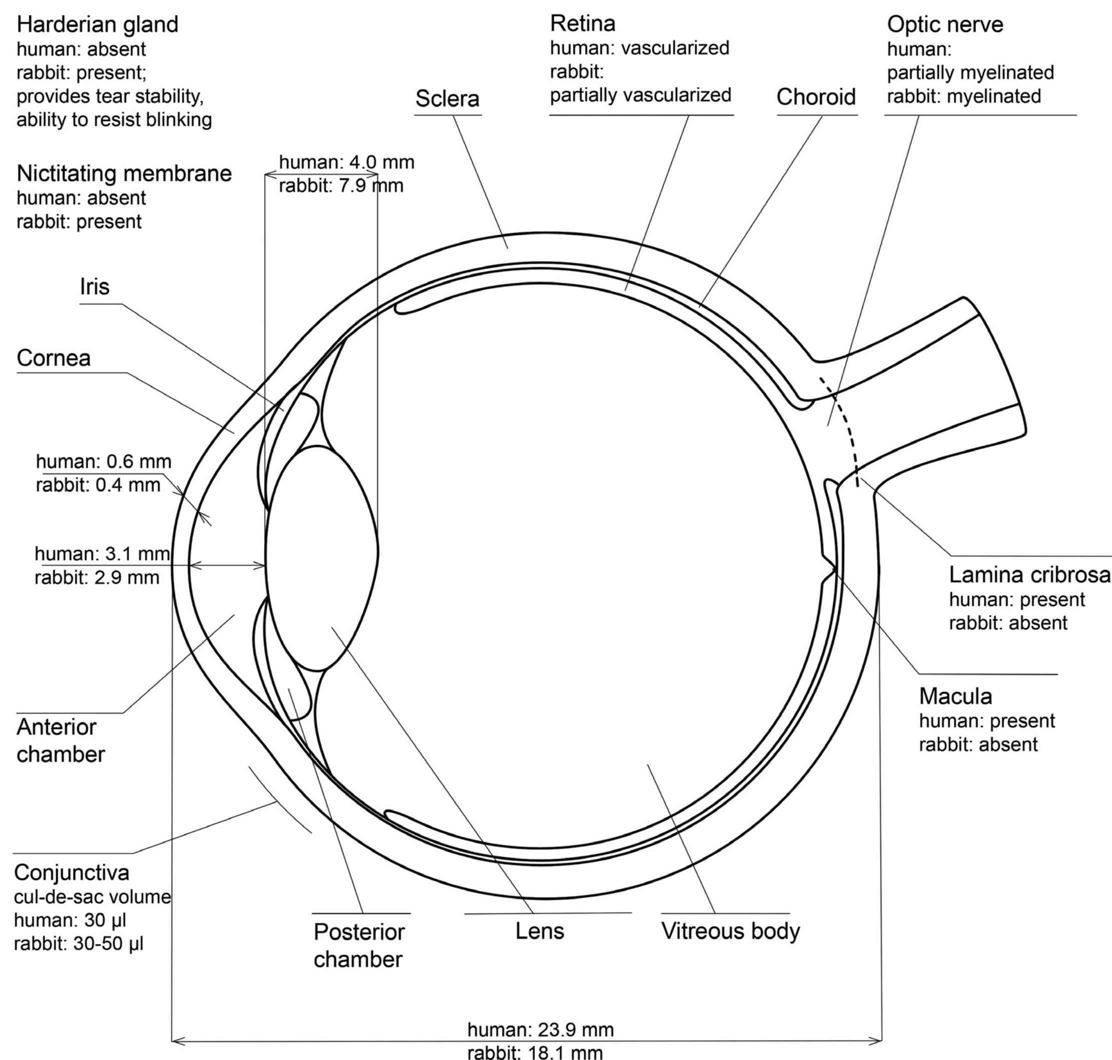

Fig. (1). Schematic diagram highlighting anatomical and dimensional similarity of human and rabbit eye. Ocular structures, which are present in rabbits but not in humans, are indicated.

(that correlates with disease severity) [28] and reaction time in driving simulator tests [28, 29].

The tear film consists of three main layers [30]: (i) a superficial lipid layer mainly composed of wax, cholesteryl esters and some polar lipids (plays a major role in maintaining tear function), (ii) an aqueous layer (important to the maintaining of the corneal transparency) and (iii) a mucous layer (responsible for the adherence of the tear film to the cornea). Any or all of these structures may be affected in DES [31]. Another sign of DES is defective immunological defense involving T and B lymphocytes, various subtypes of reticular cells and macrophages provided by Langerhans cells and lymphatic follicles of conjunctiva. Two subtypes of DES are ascribed to the underlying mechanisms of the pathology development, e.g., aqueous-deficient dry eye disease (associated with decreased tear secretion), which accounts to approximately 10% cases of DES, and hyperevaporative dry eye disease (associated with increased tear evaporation) [32]. Dysfunctions of meibomian gland result in hyperevaporative disorders. The majority (around 80%) of cases represent mixed hyperevaporative and aqueous-deficient forms of the disease [33].

Etiology of DES

DES may be triggered by multiple factors such as autoimmune disorders, meibomian gland dysfunction, a drop in systemic androgen levels, drug treatment and contact lenses wearing, environmental conditions etc. Some of these factors induce activation of T-cells targeting lacrimal glands, thus affecting their innervation, and resulting in an autoimmune response, inflammation and ultimately causing destruction of the lacrimal glands [34, 35]. Among autoimmune diseases, DES commonly accompanies Sjogren's syndrome, a chronic rheumatic disorder characterized by destruction of the glandular tissue [36]. In this case DES is associated with lymphocytes infiltration and inflammatory damage to the eye. The corneal and conjunctival epithelial cells are stimulated to express inflammatory molecules such as cytokines and cell adhesion molecules [37, 38], which promote lymphocyte activation

and their recruitment to the eye tissues and lacrimal glands [39]. As a result, inflammation occurs within the lacrimal functional unit affecting tear quantity and composition. Besides the Sjogren`s syndrome, this mechanism may develop in patients with Graves-Basedow's disease, diabetes and various infections (Epstein-Barr virus, herpes simplex virus-1, hepatitis C virus, and Bacillus xerosis) [40-43]. The lacrimal secretion may be affected by several pharmaceuticals (acetylsalicylic acid, atropine, antiestrogen tamoxifen, antineoplasic busulfan, beta-adrenergic blockers, contraceptives, tranquilizers), as well as some toxic chemicals (s.a. botulinum toxin) [41, 44, 45]. Other causes of DES include neurosensory deprivation of the lacrimal basin caused by the trigeminal nerve damage. The corneal damage resulting from dryness, incomplete blinking, traumatic aggression or lack of neurotrophic stimuli induces release of pro-inflammatory neurotransmitters enhancing neurogenic inflammation symptoms [46]. Finally, changes in lipid layer of the tear film result in increased evaporation of tears and, consequently, in many of the signs and symptoms of chronic DES. Thus, obstruction of the meibomian gland may decrease secretion of tear lipids. In addition, lipid composition of the tear film may be degraded by lipases originating from bacteria present in the eye lid [47, 48].

Rabbit Models of DES

Denervation

The model is based on the finding that main lacrimal gland and conjunctival goblet cells are under control of parasympathetic nerve stimulation [49]. Consistently, DES may be induced by surgical removal of the greater superficial petrosal nerve (GSPN). Indeed, the section of 5 mm portion of GSPN resulted in a rapid onset of DES in rabbits, persisting for at least seven days [50]. On the first day after the operation a decreased tear flow was reported in a standard Schirmer's test. The intense staining of the conjunctiva by rose bengal dye indicates a breakdown of the protective tear film. Increased frequency of blinking, characteristic dry spots on the mucosal surface of an eye and the reduced number of goblet cells are also observed. In summary, this model exhibits most of DES symptoms observed in humans, especially in Lambert-Eaton syndrome, associated with the dysfunction of the parasympathetic innervation of lacrimal glands [51]. Recently, partial transplantation of the main lacrimal gland from a healthy eye of the same animal was performed on rabbits [52]. The study has identified a prospective approach for treatment of this condition.

In the alternative model, DES is induced by a mechanical corneal nerve injury. It is a frequent complication of eye surgery, such as laser vision correction, which diminishes corneal sensitivity, weakens the tear secretion reflex and, therefore leads to the post-operational DES. Novel treatments, including nerve regeneration agents, are currently under trials using this model [53].

Application of Pharmacological Compounds

The pharmacological model of DES relies on the fact that ocular surface tissues (cornea, conjunctiva, accessory lacrimal glands, main lacrimal glands and meibomian glands) are connected by neuronal reflex loops that may be inhibited by antimuscarinic drugs, such as atropine [54-56]. Indeed, topical administration of 1% atropine sulfate into the lower conjunctival sac of each eye three times a day results in development of major DES symptoms including dry spots on the surface of the cornea and decreased tear production starting from the second day of treatment [57, 58]. This model is primarily used for preliminary screening of synthetic tear substitutes [57], as well as various anti-inflammatory drugs [56, 58, 59].

Application of Toxic Compounds

This method is based on damaging effects of a topical antiseptic benzalkonium chloride on eye surface and, in particular, epithelial goblet cells. Benzalkonium chloride is a preservative found in some anti-glaucoma medications. Application of this compound was shown to make tear films unstable, resulting in a loss of its protective function towards epithelium of an eyeball. Furthermore, benzalkonium chloride stimulates overexpression of inflammatory mediators, such as ICAM-1, or interleukins IL-6, IL-8 and IL-10, capable of inducing apoptosis in epithelial goblet cells. The latter are responsible for production of mucin proteins – gel-forming components of the mucous portion of tears. In rabbit models, a five-week course of benzalkonium chloride instillations leads to development of well-pronounced DES symptoms, including eye redness and inflammatory cell infiltrations [60-62]. The described effects mimic DES in Sjorgen syndrome patients [63, 64]. Histological studies reveal a decrease in goblet cell density and ultrastructural disorganization of both cornea and conjunctiva [62]. Loss of goblet cells results in mucin deficiency, detected by immunofluorescent staining of conjunctival cryosections [61]. Overall, benzalkonium chloride-induced DES provides a feasible approach for investigating the unwanted side-effects of antiseptics used in glaucoma treatment.

Induction of Autoimmune Dacryoiditis

Experimentally induced autoimmune inflammation of lacrimal glands represents another approach to studying DES associated with Sjorgen syndrome. The autoimmune response is induced by co-culturing rabbit lymphocytes with isolated epithelial cells from the lacrimal gland, removed from the same experimental animal [65, 66]. The stimulated lymphocytes are then injected into an intact lacrimal gland. Over the course of 4 weeks, a two-fold reduction of tear flow and severe corneal damage can be observed. The diagnosis of autoimmune dacryoiditis was confirmed by a histological examination of the gland after 8 weeks from the onset [66].

Direct Evaporation of Tears

Corneal surface cells can be also damaged by a tear film drying. In this model eyes of experimental animals are mechanically forced open for 1-3 hours. The experimental procedure is not time-consuming and it is easily reproducible allowing quantification of the damage caused to the cornea using methylene blue staining of a desiccated eye. The intensity of staining is known to correlate with the loss of the continuity of epithelial cells. After two hours some characteristic for DES spots are observed, and epithelial

thinning on the epithelial surface of the cornea can be detected by scanning electron miscroscopy [67, 68]. The model responds well to drugs, such as artificial tears, and it can be applied for preclinical trials.

Currently existing rabbit models adequately represent different pathogenic mechanisms of DES, even though none of them fully reflect chronicity and complexity of the disease. Unfortunately, the presence of the Harderian gland in rabbits, which is absent in human adults, influences their tear composition [2], making the results obtained from these models hard to interpret. Nevertheless, future studies in rabbits may involve mimicking other immune, endocrine, neuronal and environmental factors, crucial for the development of DES.

GLAUCOMA

Currently glaucoma as an optic neuropathy is a leading cause of blindness and visual impairment [69]. It is a multifactorial eye disease characterized by progressive damage and degeneration of the optic nerve and deterioration of visual field caused by the apoptosis of retinal ganglion cells (RGC) [70]. Numerous metabolic, functional, genetic and environmental factors have been shown to contribute to development and progression of glaucomatous neuropathy, of which elevated intraocular pressure (IOP) is considered to be the most important [71]. Other possible causes include glutamate-induced excitotoxicity [72] and autoimmunity [73]. However, in spite of all knowledge accumulated on the matter, the exact mechanism of the disease remains unclear. One of the hypotheses is based on the model in which the increased IOP alters ocular blood flow, resulting in retinal ischemia, triggering the apoptotic death of RGC [74, 75]. This assumption is strongly supported by existing evidence of RGC susceptibility to ischemic damage and reperfusion injury in different cases of experimentally induced hypoxia [76-79]. Furthermore, elevated expression of hypoxia-induced transcription factor HIF-1α, which is tightly regulated by the intracellular redox potential and is essential for neovascularization and survival of tissue regions with impaired oxygen delivery [80], was observed in patients with glaucoma [74]. In animal models two hours of induced ocular ischemia resulted in a loss of 99% of total RGC in the course of following three months [77, 79]. It should be taken into account that excessive IOP is a prevalent complication after eye injury, intravitreal injections or surgical intervention [81-84]. Thus, development of a secondary glaucoma remains a major risk for patients undergoing vitreoretinal surgery [85].

The pathomorphological studies of glaucoma in humans are hindered by limited availability of affected retinas, especially from early stages of the disease. This called for development of a wide range of relatable animal models in order to fully understand the pathophysiology of glaucoma and suggest new therapeutic approaches [2, 86]. Here we will focus on the types of glaucoma that have been reproduced and studied in rabbits.

Primary Open Angle Glaucoma (POAG)

Primary open angle glaucoma (POAG) is considered to be the most common form of glaucoma. It usually affects adults and proceeds without noticeable symptoms, slowly and irreversibly decreasing the patient's vision field [87]. POAG is characterized by increased IOP, acquired atrophy of the optic nerve and retinal ganglion layer degeneration [88]. Ocular hypertension can be caused by the failure of aqueous humor drainage from the anterior chamber through trabecular meshwork and Schlemm's canal [89]. At this point several rabbit models were used to study POAG [2].

Steroid and Adrenalin-Induced POAG

This condition is usually mimicked by a topical injection of steroids, in accordance with the fact that administration of glucocorticosteroids in humans results in ocular hypertension [90, 91]. In early study, weekly subconjunctival injections of 4 mg betamethasone to rabbits repeated over 3 weeks allowed to create a stable, reproducible and reversible IOP elevation, which was generally well tolerated by animals and responded to treatment by pressure-lowering anti-glaucoma drugs [92]. Rabbit eyes with steroid-induced ocular hypertension were histologically examined and abnormal accumulations of acid mucopolysaccharides in the corner of the anterior eye chamber adjacent to aqueous plexus (rabbit functional analogue of Schlemm's canal) and in vacuoles of trabecular endothelial cells were identified [93]. Several types of steroids were used in these studies, including cortison acetate, hydrocortisone, betamethasone, dexamethasone and prednisolone [92-94]. Even though the therapy was applied to a single eye, its effects were bilateral [93]. In most cases IOP reached maximum after 3 weeks of treatment and returned to normal during the course of the 5th week, when the animals have adapted. However, some rabbits treated with dexamethasone developed especially severe cases of ocular hypertension, persisting as long as the injections continued [93]. More importantly, prolonged steroid treatment in rabbits was often accompanied by toxic adverse effects, such as renal and adrenal dysfunction, cataracts and inflammation. Such side effects undermine the general plausibility of steroid-induced glaucoma models. Furthermore, IOP in rabbits is sometimes found to vary under stress, which makes it difficult to interpret [86]. Nevertheless, consistency and low cost of steroid-induced glaucoma models make them compelling to the present date [95]. More recently, optic nerve morphology was characterized in rabbits with adrenalin-induced POAG. The experimental animals exhibited decrease of thickness of the nerve fiber and more than 50% reduction of the optic nerve disc diameter [96]. These abnormalities were found to be comparable with those in human patients.

Chymotrypsin-Induced POAG

Eye treatment with chymotrypsin represents alternative way for experimentally elevating IOP in rabbits. Alpha-chymotrypsin is injected into the posterior chamber of the animal eye and damages the ciliary body, namely the suspensory ligaments of the lens [97, 98]. As a result, the trabecular meshwork becomes jammed by lysed zonular fibers, which over the course of seven months leads to buphtalmia, stable IOP increase, retinal degeneration and excavation of the optic nerve disc [99]. The latter indicates the loss of optic nerve head fibers.

Hydroxypropyl Methylcellulose-Induced POAG

Lately, another rabbit POAG model has been developed by injections of hydroxypropyl methylcellulose, a viscoelastic polymer that is used in anterior chamber surgery. Indeed, viscoelastics are generally known to provoke post-operational IOP increase [100, 101]. In this model, 2% hydroxypropyl methylcellulose applied twice a week for five weeks induces chronic, moderate IOP elevation, caused by violation of aqueous humor dynamics, which persists for six months [102]. According to histological studies, all eyes treated with hydroxypropyl methylcellulose develop optic nerve cupping, RGC loss and retinal detachment. In addition, numerous morphological changes typical for human cases of glaucoma are observed. These include general eye ball enlargement, lens swelling and thinning of the inner limiting membrane between the retina and the vitreous body [102]. The model allows authentically reproduce prolonged and chronic factors of POAG development and proves suitable for testing drugs with systemic effects, such as mitochondria-targeted antioxidants.

Primary Angle Closure Glaucoma (PACG)

Primary angle closure glaucoma (PACG) or acute glaucoma shares many traits with POAG but is particularly associated with the obstruction of the trabecular meshwork by peripheral iris bunching [87]. In this form of the disease aqueous humor is blocked from reaching the anterior chamber. As a result, IOP increases dramatically which may be accompanied by a mid-dilated pupil, a red eye, or nausea and vomiting. Although this is a common picture of PACG, cases without prominent symptoms are also described. Rabbit models of PACG employ either water-loading [103-105] or laser treatment of the trabecular meshwork [106, 107]. Water-loading tests are usually carried out *via* orogastral intubation, followed by significant IOP rise diminishing after approximately one hour. Despite the briefness of this condition, such model has been widely used to assess both preventive and corrective effects of anti-glaucoma drugs [104, 105]. The advantage of water-loading is that it is not invasive to the eye and therefore produces no irritation which could affect baseline IOP [105]. Still, the duration of IOP increase is insufficient in comparison to acute PACG attacks in humans [87]. Alternatively, the ablation of the trabecular meshwork with argon laser results in more stable ocular hypertension, which lasts for a few weeks and leads to buphtalmia and optic nerve cupping [106]. Yet, iridocorneal angle in rabbits is narrower than in humans significantly complicating precise laser targeting [107]. As a result, the success rate for this type of PACG induction is relatively low for rabbit eyes (about 18%). Furthermore inevitable damage to the ciliary body occasionally provokes iris adherence to the cornea and inflammation rather than expected trabecular meshwork blockage [107]. To sum up, for most researchers the complexity of this model outweighs its benefits.

Primary Congenital Glaucoma (PCG)

Primary congenital glaucoma (PCG) is associated with abnormal development of the anterior chamber angle. Mutations in gene loci *CYP1B1* and *LTBP2* are currently known to be associated with PCG development in humans, but their precise role in the mechanism of the disease remains unknown [108]. The rate of PCG in some rabbit populations (specifically New Zealand albino strain) is relatively high, which makes them a suitable model for studying this disease [109-113]. Rabbit congenital glaucoma is supposed to be a semilethal recessive condition, associated with mutations in one or more autosomal genes [109]. It is phenotypically similar to the human one being characterized by the early age of onset (3–6 months for both species), reduced outflow facility, chronic ocular hypertension, buphtalmia and optic nerve degeneration [110, 111]. Some of the aberrations in the anterior chamber morphology are also shared between the species, as was observed by electron microscopy. Thus, PCG is generally characterized by disorganization of trabecular meshwork and its partial replacement by extracellular matrix [112, 113]. It is hypothesized that mutated loci must be somehow involved in the regulation of cell differentiation in the anterior chamber angle. To shed light on the disease mechanism, the proteomics of rabbit PCG was recently analyzed by liquid chromatography–tandem mass spectrometry of aqueous humor samples [114]. The study revealed differential expression of several genes engaged in extracellular matrix homeostasis, tissue differentiation and oxidative stress, although the upstream source of these changes remains to be determined.

Despite the fact that rabbit PCG has provided valuable knowledge for elucidating of fundamental mechanisms of glaucoma and is known to response to treatment with human anti-glaucoma drugs [115], the limitations of this model should not be disregarded. To begin with, rabbit anterior chamber and outflow pathway morphology significantly differs from that in humans [112, 113]. Furthermore, in contrast to human congenital glaucoma, IOP in rabbits may normalize with age for unknown reasons [111]. The limited commercial availability of specific rabbit strains prone to PCG also contributes to the list of the rabbit model disadvantages. Finally, no mutations in the *CYB1P1* or *LTBP2* genes of the affected animals were identified so far [114]. Nevertheless, since rabbit genome sequence is now available new possibilities for studying genetic background of glaucoma are emerging.

Normal Tension Glaucoma (NTG)

Normal tension glaucoma (NTG) strongly resembles open angle glaucoma except it lacks the distinctive IOP rise. Insufficient blood supply of a retina and the optic nerve, as well as autoimmune response are presumed the main neurodegenerative factors in NTG [116, 117]. In animal models retinal ischemia, which is innate to this disorder, can be reproduced by usage of vasoconstrictors, such as endothelin-1 (ET-1). ET-1 is a 21 amino acid long peptide associated with a number of renal, ocular and cardiovascular disorders in humans. In rabbits, intravitreous injections of ET-1 have been shown to cause obstruction of retinal vessels [118-122]. In these experiments tissue blood velocity was measured by laser speckle contrast imaging [123] and the alterations in blood vessel diameter and optic nerve disc size were estimated from fundoscopy. Numerous studies report that the administration of picomolar concentrations of ET-1

to rabbits leads to persistent reduction of blood stream velocity and the diameter of retinal arteries [119, 122], whereas higher doses cause their complete occlusion [118, 121]. Histological examination of the affected eyes revealed axonal loss, demyelination of the intraocular region of the optic nerve, gliosis and hypertrophy of connective tissue [119, 120]. This model is feasible for studying effects of hypoxia and reperfusion injury in glaucoma but its strongest disadvantage is the possibility of unwanted side effects of ET-1, which is able to diffuse to other tissues. It is also important to take notice that in contrast to humans the intraretinal portion of the rabbit optic nerve is myelinated [124]. Thus, some evidence obtained from this model can be roughly adequate to neurodegenerative changes in the course of human glaucoma.

In summary, throughout several decades of research rabbit models proved to be an irreplaceable tool for the study of pathophysiology of glaucoma and pharmacological screening for potential neuroprotective agents. The size and overall anatomy of the rabbit eye is similar to the human eye, which facilitates injections and surgery, though some morphological nuances should not be overlooked [107, 124]. The natural frequency of glaucoma in certain rabbit populations gives an opportunity to collect data by using same approaches and instrumentation as with human patients without resorting to genetic manipulations [111]. Furthermore, some models of induced glaucoma, such as the water-loading test, while exceptionally cheap and easy to perform, provide a robust, quick and consistent response [105]. Even though some researchers consider the rabbit a sub-optimal model for glaucoma study [86], it remains the most convenient choice among larger non-linear mammals.

AGE-RELATED MACULAR DEGENERATION

Development of animal models of AMD is another challenging issue currently being investigated. Given a number of recent reviews on the subject [3, 5, 21], here we will only focus on the molecular mechanisms and major clinical manifestations of AMD as well as the strategies of their simulating in animals with focusing on rabbit models.

AMD is often a cause of a visual impairment in individuals over 65 years old. The first signs of AMD may be revealed funduscopically and histologically, including thickening of Bruch`s membrane, development of subretinal deposits such as drusen or pseudodrusen, and pigmentary changes in RPE [125]. During the early stages AMD is generally not associated with the loss of visual acuity, only manifesting in a decrease in dark adaptation [126, 127]. However, epidemiological studies indicate that patients with signs of early AMD have an increased risk of its progression into the late forms [125]. Advanced disease is classified into non-neovascular (non-exudative or 'dry') and neovascular (exudative or 'wet') forms [3, 5, 21, 128]. Dry AMD manifests as geographic atrophy that is represented by a loss of RPE cells and associated degeneration of overlying photoreceptors. The development of wet AMD includes growth of blood vessels from the choroid that penetrate the Bruch`s membrane and invade the retina (choroidal neurovascularization, CNV) resulting in retinal hemorrhages. These late forms of the disease differ by degree of incidence and prognosis in respect to the visual function. Wet AMD was shown to be a less frequently occurring form, manifesting in older people, however it is characterized by the greatest risk of development of severe visual impairments. Yet, this is the only form of the disease for which efficient therapeutic strategies have been developed [21, 125].

Mechanisms of AMD Development

Growing evidence indicates that AMD is a complex disease caused by metabolic, functional, genetic and environmental factors. The major pathogenic event in early AMD is the formation of pathological deposits – large 'soft' drusen (in contrast to small 'hard' drusen minor accumulation of which is associated with normal aging) located within the Bruch`s membrane and/or between Bruch`s membrane and RPE. One of the sources of these deposits were suggested to be PRE cells which secrete partially digested membrane-bound proteins and lipids towards the Bruch`s membrane. These lipids may also originate from photoreceptor discs consumed for degradation [129, 130]. Complement proteins, immunoglobulins, apolipoproteins B and E, fibrinogen, vitronectin, amyloid β and various lipids including oxidized ones were revealed as components of drusen indicating involvement of immunoinflammatory, oxidative and lipid metabolic pathways in their formation [130-133]. Notably, the predisposal role of immune system alterations in AMD development was confirmed by genetic studies revealing single nucleotide polymorphisms in genes encoding complement components, chemokines and their receptors. Expression of these altered genes was shown to correlate with increased risk of the disease [134]. In addition, light exposure, environmental factors and oxidative stress were shown to trigger AMD pathogenesis [135-137]. Another hallmark of the early AMD is thickening of the Bruch`s membrane which is likely to be caused by single nucleotide polymorphisms in *HTRA1* and cholesterol-related genes regulating protein and lipid composition of the membrane [5]. Development of the above-mentioned abnormalities induce further progression of AMD into the late dry or wet forms. Accumulation of drusen between the Bruch`s membrane and RPE disturbs the chorio-RPE transport resulting in a degradation of the RPE cells and photoreceptors [21]. An RPE damage is also induced by accumulation of lipofuscin and by-products of the retinoid cycle such as A2E (the product of the reaction of two molecules of all-trans-retinal with ethanolamine) that kills cells *via* oxidative mechanisms [138]. Finally, RPE cell death in AMD is induced by a cascade initiated by *Alu* RNA accumulation due to down-regulation of endoribonuclease DICER1 by the oxidative stress [5]. The loss of RPE cells manifests as regions of hypopigmentation and represents dry AMD. Thickening and accumulation of drusen within Bruch`s membrane contributes to its degradation that consequently evokes vascularization of the retina (CNV) resulting in hemorrhages and development of wet AMD. The CNV was suggested to be induced by disruption of the balance between proangiogenic (vascular endothelium factor A, VEGF) and antiangiogenic (antiangiogenic pigment epithelium-derived growth factor) factors normally secreted by RPE cells [21].

Strategies of Simulating AMD Using Animal Models

Several reasons account for hindered development of animal AMD models. Firstly, the complex nature of the disease makes it necessary to simultaneously model several etiological factors. Secondly, differences in the anatomical organization of the retina in humans and various animal species, occasionally lacking the macula. As a result neither of the animal models can fully reproduce the human disease [21]. The non-human primates such as Macaque and Rhesus monkeys were shown to be the only animals that can develop early signs of AMD including drusen formation with similar composition and location to humans. However no signs of advanced disease, i.e. geographic atrophy or CNV have been demonstrated for primates [5]. Some features of early-onset and advanced AMD can be reproduced in rodent models. The main advantage of using rodents is their lineage and availability for genetic manipulations, thus making it possible to study the impact of genetic factors on the etiology of AMD. Therefore, rodent models are often used for modeling genetic alterations in the complement pathway, oxidative damage and/or metabolic pathways alterations [3]. Although rodent models are commonly used in AMD studies, their applications are mostly limited to the dry form of the disease since only few mouse strains show progression of the pathological process from Bruch`s membrane thickening and RPE/photoreceptor damage to CNV [5].

Rabbit Models of AMD

Given the limitations of primate and rodent AMD models, there is a need to develop alternative animal models, capable of efficiently reproducing the signs of the early-onset and the neovascular forms of AMD. Furthermore, it is sometimes desirable to investigate AMD in larger eyes, for instance in studies aimed at development of AMD therapeutic strategies. Various rabbit models can be utilized for these purposes. Although rabbit eye is unique in that the retina does not possess distinct macula and contains superior central bundle of vessels, it is substantially larger than the rat/mouse eye and is more similar in size to the human eye. Furthermore, blood vessels in rabbit retina are more superficial and therefore it is much easier to reach them with injections. These anatomical features make rabbit eyes suitable for manipulations evoking AMD development, e.g., intravitreal and subretinal injections of substances, which induce CNV, or a surgical rupture of the Bruch`s membrane. In addition, rabbit eye trials are often used in ocular pharmacology and the corresponding AMD models are well suited for monitoring of anti-neovascular and neuroprotective drug delivery, as well as for assessment of new AMD treatment approaches such as RPE transplantation or laser, X-ray and photodynamic therapy. At present, several rabbit models of early and advanced stages of AMD have been developed.

Early-Onset AMD Models

In early studies researchers attempted to induce drusen-like changes in Bruch's membrane by subretinal injection of vitreous removed from one rabbit eye and injected beneath the retina of the fellow. The vitreous autotransplantation resulted in RPE budding followed by formation of a drusen-like material located within the Bruch's membrane. The parent RPE cells displayed associated degenerative changes including a loss of pigment granules, an accumulation of phagolysosmes, lipofuscin granules and residual bodies, an expansion of smooth-surfaced endoplasmic reticulum, vacuolation and disappearance of mitochondria. These changes are similar to those observed during drusen formation in primates [139]. More recently, the biogenesis of drusen was simulated in rabbits *via* a subretinal implantation of lipofuscin mimicking particles composed of albumin-based glycoxidation products. The eyes implanted with these particles produced drusen-like deposits and in some cases displayed signs of sub-RPE choroidal neovascularization [140]. Furthermore, the manifestations included abnormal fundus autofluorescence patterns similar to those observed in aging human eyes with or without AMD [141].

Considering a role of lipid metabolic pathways in AMD pathogenesis, some signs of the early-onset AMD in rabbits were developed in response to a cholesterol-enriched diet. Indeed, animals exposed to excess of cholesterol exhibited alterations in retinal structure similar to those observed in human AMD including changes in the RPE cells (accumulation of dense bodies, debris from cell membranes, vacuoles and clumps of lipids) and a thickening of the Bruch's membrane. In this model, the retinal neuroglia such as Müller cells and astrocytes was also affected [142]. Notably, the cholesterol-induced model was recently characterized by identification of another AMD hallmarks such as formation of drusen-like debris, increased level of β-amyloid and increased generation of reactive oxygen species. The latter seems to be due to a contribution of oxidized cholesterol metabolites to the AMD pathogenesis [143].

Models of CNV

Development of CNV in rabbits was observed in early studies as a complication of laser photocoagulation AMD treatment. Indeed, laser photocoagulation not only occludes the existing choriocapillaries and prevents subretinal hemorrhages (SH), but can also produce neovascularization, particularly when the Bruch's membrane is diseased [144]. Laser photocoagulation-induced CNV model was developed in primates, but it was associated with extensive retinal damage. Therefore, an attempt was taken to develop a rabbit model where argon laser endophotocoagulation was applied to minimize retina injury. The argon photocoagulation did not cause clinically apparent CNV associated with leaking of fluorescein and was restricted only to microscopic neovascularizations [145].

Currently, the common approach for induction of CNV in rabbits involves subretinal delivery of proangiogenic agents *via* injections or by implantation of their complexes with various supporting matrices in the so-called controlled release systems that efficiently deliver small amounts of the agent. The bioactive compounds extensively used for the CNV stimulation include linoleic acid hydroperoxide (LHP), basic fibroblast growth factor (bFGF) and VEGF.

The age-dependent increase in lipid peroxidation in human macula and Bruch's membrane induces CNV and therefore it plays a role in AMD pathogenesis. Consistently, subretinal injections of peroxidized lipids such as LHP are used for CNV modeling in animals. Substantial amounts of

LHP (>100 µg) being delivered to subretinal space of the rabbit eye after two weeks initiated a development of histologically detected CNV in 46% of animals with fluorescein leakage in 9-15% of the animals [146]. In alternative study the angiographically visible and histologically verified CNV was induced using the same approach in two weeks in 27% of cases. The neovascularization was associated with a damage of the outer retinal layers [147].

LHP-stimulated neovascularization is associated with up-regulation of several pro-angiogenic cytokines [148], which are also widely applied for CNV induction in the rabbit AMD models. Subretinal implanting of bFGF-impregnated gelatin microspheres prepared by forming a polyion complex between gelatin and bFGF in two weeks resulted in development of CNV and fluorescein leakage from new vessels that persisted up to 8 weeks [149]. The CNV membranes in this model were primarily composed of the RPE cells and vascular endothelial cells thereby resemble those found in the CNV membranes surgically excised from patients with AMD [150]. The more prolonged and extensive model of wet AMD was developed in rabbits following subretinal injection of a cocktail containing bFGF and lipopolysaccharide incorporated in Heparin-sepharose beads. Two phases of CNV were observed in this case. The primary phase representing neovascularization in the subretinal space associated with an injury of the Bruch's membrane was observed in 2 weeks and kept stable for 3 months. The secondary phase was characterized by neovascularization in the sub-RPE space away from the initial injection related injury and developed for 8 months. Both phases demonstrated leakage throughout the entire observation period [151].

It is known that angioproliferation in AMD is induced by RPE cells-secreted proangiogenic factor VEGF [21], which therefore is widely used for CNV simulation along with bFGF. The injections of VEGF mixed with matrigel into the rabbit subretinal space initiates a growth of new subretinal blood vessels. The angiographic leakage was observed 1 week after injection, increased at weeks 2 and 4 and persisted till week 9 [152]. An alternative approach for delivery of VEGF to the rabbit retina includes transfer of its gene using adenoviral vector. Normally, adenoviruses induce immunogenic properties but immune privilege of the eye tissues limits the immune response against the vector. Adenoviral vector can be injected either intravitreally or into the subretinal space of the rabbit eyes. In the first case overexpression of VEGF causes a new vessel formation in six days after injection although neovascularization and structural changes in the retina are concentrated on the optic nerve head but not at periphery [153]. In the second case the CNV with leakage observed in 2-4 weeks is more pronounced. The choroidal endothelial cells proliferate and form pathological vessels in the subretinal space, which is accompanied by a macrophage stimulation, retinal edema, and a loss of photoreceptors. Notably, in this model an increased expression of bFGF is observed confirming a role of both factors in the CNV induction [154]. Consistently, intravitreal implantation of 1:1 mixture of VEGF and bFGF in Hydron polymeric pellets results in more rapid dilation of blood vessels (48 h) spreading over the entire optic disk and medullary rays (4-7 days) with further hemorrhages and retinal detachment (14 days) [155].

Subretinal Hemorrhage Models

A prevailing complication of the neovascularization in wet AMD is SH resulting in a loss of central vision. The blood exposure produces toxic effects on the retina by two mechanisms. Firstly, it is mechanical impacts such as fibrotic shearing of photoreceptors, hypoxia and metabolic disruption imposed by the clot as a diffusion barrier. Secondly, blood components produce direct neurotoxic effects [156]. To assess these pathological processes, rabbit models of SH are extensively utilized. The most common approach for simulating SH in rabbits is an injection of fresh autologous blood into the subretinal space [156-158]. The ophtalmoscopic and microscopic studies after the injection revealed damages of photoreceptors and irreversible retinal destruction within 24 hours [157]. More moderate experimental SH can be induced in rabbits by injection of balanced salt solution into the subretinal space followed by puncture of the choroid [159]. Finally, the blood can be injected under the RPE, which results in a low, diffuse sub-RPE hemorrhage [156]. These rabbit models are used in particular for searching of possible noninvasive approaches for suppression of SH consequences alternative to surgical removal of submacular blood in AMD.

Trialing of Drug Delivery and Treatment Strategies Using Rabbit Models of AMD

As noted above, rabbit models of AMD are well suited for testing various treatment strategies against the disease. Early works describe development of laser photocoagulation approaches for aborting CNV/SH consequences. The corresponding rabbit models were used to examine laser types most relevant for the treatment. Thus, experimental retinal photocoagulation in rabbits with diode laser was found to be less harmful than with argon laser [160]. These results were confirmed in subsequent study comparing efficacy of diode, krypton and argon lasers for choroid photocoagulation in rabbit model of SH. It was demonstrated that laser light penetration increases at longer wavelengths and diode infrared laser was admitted suitable for coagulation of choroid without affecting the overlying retina [161]. Rabbit model of bFGF-induced CNV was used to trial feasibility of X-ray therapy for treatment of wet AMD. In this study the irradiated eyes display decreased fluorescein leakage from the CNV lesions associated with low degree of vascular formation even four weeks after the exposure indicating that radiation therapy might be beneficial in control of CNV [162]. Finally, rabbit AMD models including LHP-induced CNV were applied in development of more advanced AMD treatment approach namely photodynamic therapy. These studies involve standardization of light application parameters and trialing feasibility of various photosensitizers [147, 163]. Owing to their relatively large size rabbit eyes are suitable for surgical manipulation practices particularly in development of transplantation techniques for AMD treatment. These include for instance macular translocation by radial scleral outfolding and transplantation of autologous iris pigment epithelium into the subretinal space [164, 165]. Feasibility of the latter approach

for AMD treatment was confirmed using rabbit model of the disease induced by mechanical debridement of the Bruch membrane [166].

Another important line of AMD research employing rabbit models includes trials of new pharmaceuticals designed for treatment of predominantly wet forms of the disease. These trials comprise efficacy response evaluation and monitoring of delivery of such pharmaceuticals as well as examining of their safety, pharmacokinetics and pharmacodynamics. The common approach in AMD medication is intravitreal injections of anti-neovascular and neuroprotective compounds. Thus, intravitreally injected tissue plasminogen activator in rabbits with experimental SH results in rapid lysis of subretinal blood clots but does not diminish retinal damage [158]. By contrast, the injections of anti-VEGF drug triamcinolone acetonide reduce SH-inducedretinal toxicity preventing photoreceptor loss and apoptosis [156]. Recent preclinical study revealed that weekly intravitreal injections of integrin $\alpha 5\beta 1$ inhibitor JSM6427 results in inhibition of CNV in rabbit model induced by implanting VEGF/bFGF pellets [167]. An alternative to the direct injections of anti-AMD compounds is their delivery *via* adenoviral gene transfer. This approach was successfully applied for instance for transfer of Heme oxygenase-1 into rabbit ocular tissues by microinjections of its recombinant replication-deficient adenovirus cDNA into the anterior chamber, vitreous cavity, and subretinal space. The increased activity of the enzyme in ocular tissues was suggested to protect against oxidative stress that contributes to AMD pathogenesis (see hereinbefore) [168]. It was demonstrated also that model CNV induced in rabbits by intravitreal adenoviral gene transfer of VEGF can be prevented by 15-lipoxygenase-1 delivered using the same approach. The activity of the latter blocks the expression of VEGF and associated receptors thereby inhibiting neovascularization [169]. Another prospective approach for delivery of macromolecules to the posterior segment of eye is transscleral iontophoresis feasibility of which was widely studied using rabbit models (see for example [170, 171]). Considering the obtained data it was suggested that the transfer of anti-neovascular drugs in AMD using this method is a safe alternative to their intravitreal injections that are characterized by increased risk of promoting cataracts, hemorrhages, retinal detachments and other side effects [172].

Overall, the growing evidence indicates significant importance of employment of rabbit models in AMD research. Rabbit eyes are feasible for simulating and studying of both early and advanced stages of the disease. Furthermore, rabbit models have pronounced practical application being well suited for development of new strategies for the treatment of pathological angiogenesis and other sings of AMD.

LIGHT-INDUCED RETINAL DAMAGE

Investigation of the mechanisms of light-induced retinal damage (photochemical damage, PD) and associated visual dysfunctions (light-induced retinopathies, AMD) mostly rely upon use of rodent and primate models. However, recent studies based on use of the rabbit models of retinal PD demonstrated their viability in detailed characterization of the process at molecular level [173, 174] and in biomedical applications [173, 175, 176]. Here, we will focus on early and recent rabbit models of the retinal PD, beginning with brief description of clinical, morphological and mechanistic aspects of the light-induced retinal damage in various species, based on few excellent reviews on the topic [177-181].

The prolonged eye exposure to natural light arising from sun and to light from other sources increases risk of the retinal PD, which is associated with progression of light-induced retinopathies and other visual dysfunctions, including AMD [135, 177]. Firstly, direct sungazing causes a solar retinitis (SR), which is characterized by post-exposure injury to the RPE cells, whereas photoreceptors are mostly not affected, but exhibit mild tubulovesicular changes of the outer segments and formation of microtubular aggregates in the inner segments. Some of the patients may completely recover from the injury, since RPE cells are capable of rapid regeneration and photoreceptors can gradually regenerate their outer segments. However, high irradiation doses are associated with the pathological processes leading to complete degeneration of the photoreceptors and the respective development of permanent central scotomas [177]. Secondly, exposure to the light from ophthalmological instruments such as operating microscope may cause an iatrogenic retinal insult, which is mechanistically similar to SR, but the lesions affect both photoreceptors and RPE cells in this case [177]. Photic maculopathy is the intermediate condition observed following eye illumination with an indirect ophthalmoscope, which affects both photoreceptors and RPE cells and results in chronic long-lasting degenerative processes in the retina, such as impaired outer segment production, photoreceptor loss, RPE depigmentation and CNV. The two latter symptoms are characteristic to AMD, described in the previous section. The factors favoring the light-induced retinal injury causative AMD include white race, light-colored irides, aphakia and pseudophakia, whereas nuclear opacity of the lens (cataract) reduces risk of the disease [177]. Finally, retinal PD can be a serious complication of the visual disorders affecting functioning of photoreceptor and PRE cells. These are predominantly hereditary genetic disorders such as recessive Stargardt's macular dystrophy, Best disease, stationary night-blindness, Oguchi disease and retinitis pigmentosa [178].

Mechanisms of Light-Induced Retinal Damage

Retinal PD was divided into two classes differing by their action spectrum (efficiency with which electromagnetic radiation produces a photochemical reaction plotted as a function of the wavelength of the radiation) and primary targets [178, 181]. Class I damage (also known as Noell damage) is characterized by an action spectrum that resembles absorption spectrum of the visual pigment (RAS, rhodopsin absorption spectrum [181]). It appears after long exposures (>1.5 hours) to relatively low retinal irradiances (<1 mW/cm^2) of green-filtered or white light and mostly affects photoreceptors and RPE cells [178, 181]. RAS was hypothesized to occur under non-fully bleaching conditions when rhodopsin being the most sensitive chromophore in the system [181]. In addition to photopigments, possible

photosensitizers (see ref. [182]) for class I PD include retinoids, all-*trans*-retinal and all-*trans*-retinol, as well as cytochrome C oxidase, although its involvement remains controversial [178]. Consistently, the process of rhodopsin regeneration is crucial for class I PD [180]. Class II damage (also known as Ham or "blue-light" damage) has an action spectrum maximal at short wavelengths (SAS, short wavelength action spectrum [181]). It occurs under short-term exposure (<5 hours) to high irradiances of white light or laser lines and primarily targets the RPE cells [178], while UV light induces damage to photoreceptors [181]. SAS likely occurs at high retinal irradiances, ensuring bleaching of rhodopsin, so other chromophores mediate the PD [181]. The class II PD is most likely related to photosensitization of lipofuscin (a conglomerate of modified lipids and bisretinoids in the lysosomes of RPE cells) components, such as A2E and its isomers, the all-*trans*-retinal dimer series, A2-DHP-PE, *etc.* [183]. For instance, the photoexcitation of A2E leads to formation of singlet oxygen, superoxide radical anion and hydroperoxides, which cause cellular toxicity *via* oxidative stress [138, 178]. The light-induced development of oxidative stress is a common feature of the both classes of retinal PD [182]. This process is greatly facilitated in retina due to its high oxygen consumption, elevated content of long-chain polyunsaturated fatty acids in photoreceptor membranes and deficiency of reducing agents in outer segments of photoreceptors [180, 184-186]. The lowest estimate of redox potential in rod outer segments under illumination (based on comparison of *ex vivo* levels of disulfide homodimer of photoreceptor Ca^{2+}-sensor protein recoverin with redox dependence of its *in vitro* thiol-disulfide equilibrium) reaches -160 to -155 mV [174]. The alternative estimate, based on the suggestion that recoverin is mostly Ca^{2+}-loaded under prolonged illumination [187] is -190 to -180 mV. Being an integral part of retinal PD, the oxidative stress is accompanied by changes in transcriptional regulation, enzymes activity and initiation of apoptosis by various pathways [179, 180]. Overall, photic injury of retina results in damage of RPE and photoreceptor cells, followed by their apoptosis, ultimately leading to progression of visual dysfunctions. It is believed that SR develops following class II damage mechanism, while degeneration of photoreceptor and RPE cells in AMD might occur following both mechanisms [177].

Strategies for *In Vivo* Studies of Light-Induced Retinal Damage

The light-induced retinal damage has been extensively studied using various, predominantly rodent and primate, animal models [178, 180]. In fact, classes I and II of retinal PD were initially described in the works by Noell *et al.* and Ham *et al.* in rat [188] and monkey [189] models, respectively. The retinal PD is typically induced using the following exposure conditions: (i) low-level exposure of unrestrained animals to white light for periods ranging from hours to weeks; (ii) high-level exposure of unrestrained or restrained (anesthetized) animals to white light for periods ranging from 30 minutes to several hours; (iii) exposures of unrestrained or restrained animals to monochromatic light for 1-30 minutes [190].

Class I damage (Noell damage, RAS) was initially observed in albino rats in response to exposure for 1.5-3 hours to constant green light (490-580 nm) yielding retinal dose of 0.038 J/cm^2 [181, 188]. This type of PD was subsequently stimulated in mice, macaque, hamsters, fish, chickens and other species. The general approach for induction of class I PD includes prolonged (from 2-12 hours to weeks in rodent models) exposure of unrestrained animals to green, fluorescent or incandescent white light of low intensity [178]. The retinal morphology of PD varies between the species, although photoreceptors are always affected. In particular, exposure of rats for 1.5-48 h affected only photoreceptors, while exposures for 8-50 days leaded to damage of both photoreceptor and RPE cells [191]. By contrast, in hamsters, the RPE cells were damaged first, while photoreceptors were only slightly affected [192]. Generally, retinal PD in nocturnal rodents is confined to rod photoreceptors. This effect serves a basis for quantification of PD *via* studies of photoreceptor layer and oxidation state of visual proteins. The light-induced damage of photoreceptor cells displays regional differences, depending on the light intensity. Moderate PD affects central region of the retina (1-2 mm from the optic nerve head), while higher light intensities affect its periphery. PD of rod cell is initiated at distal tips of rod outer segments (ROS) and subsequently extends to entire ROS [180]. The action spectrum of class I PD in rod-dominant animals matched rhodopsin absorption spectrum, indicating targeting of the pigment. Consistently, cones of different types can be targeted in cone-dominant species by exposure to corresponding monochromatic light [178]. The genetic approaches using transgenic animals provided new evidences of the role of phototransduction and visual cycle in retinal PD. For instance, using transducin and arrestin null mice it was found that photoreceptor damage depends on phototransduction at low irradiation levels, but becomes independent of transducin activity at high light intensities [180]. Similarly, animals with a visual cycle (*rd*) mutation are more susceptible to class I damage and mice with genetically retarded rhodopsin regeneration displayed lowered extent of the PD [178, 180].

Class II damage (Ham damage, SAS) was initially observed in monkeys subjected to short (1-1000 sec) exposure to monochromatic laser lines (441.6 – 1.064 nm). The retinal irradiance for 1000 sec illumination was 30 mW/cm^2 at 441.6 nm (He-Cd laser) and gradually raised with wavelength up to 5.4 W/cm^2 at 632.8 nm (He-Ne laser) [189]. In the follow-up studies, Ham damage was reported in a number of primate, rodent and other models [178, 180]. In general, class II damage is induced under short-term (from seconds to hours) illumination of restrained (anesthetized) animals with white light or laser lines of high irradiance. The Ham damage induced by visible light causes in majority of species swallowing and inclusions in RPE, while exposure to UV and violet (320-440 nm) light is accompanied by predominant damage of photoreceptors [181].

The Ham damage induced in primate models is used for establishing of international standards for the protection against retinal damage by lasers and other light sources. By contrast, Noell damage induced in rodent models is used in most of laboratory studies owing to the well-established

roles of rhodopsin and visual cycle in class I PD and the fact that rodents are more common laboratory animals [181]. Both approaches are used in investigation of the mechanisms of photo-induced retinal degeneration, including propagation of oxidative stress and apoptosis, as well as in development of protectors against PD. Since light is a leading cause or contributing factor in certain retinal diseases (e.g. SR and AMD), these studies are of medical importance. Furthermore, animal models of PD are often used for simulation of the retinal damage induced by apoptosis. This approach may have a number of advantages such as (i) synchronized triggering of apoptosis, (ii) fast development of the response and (iii) possibility to tune the damage in terms of severity by varying light intensity or exposure duration [179].

Rabbit Models of Light-Induced Retinal Damage

Rabbit models of PD were employed in laboratory studies beginning from the middle of 70`s. The principle advantage of rabbit models for this type of applications is their feasibility for inducing class II Ham damage in contrast to rodents in which class I retinal damage is dominated. In fact, the work by Lawwill in 1973 [193] described rabbit model of PD that seems to fit to class II damage [181] three years before the classical study by Ham *et al*. performed using albino rats [189]. Although the main aim was to characterize consequences of prolonged exposure of rabbit retina to low-intensity light, the thresholds for the minimal detectable damage and cumulative light doses in Lawwill`s study were more relevant to class II Ham damage. Indeed, the rabbits were anesthetized and exposed for 4 hours to the light from broad-spectrum sources or monochromatic 514.5 mn laser with irradiations between 0.015-1.6 W/cm^2 or 0.017-0.047 W/cm^2, respectively [193]. Under these conditions the threshold for the minimal detectable damage after exposure to broad-spectrum source was 45 mW/cm^2 (cumulative dose of 648 J/cm^2) which exceeds Noell damage characteristics (<1 mW/cm^2 [181]). In the case of 514.5 mn monochromatic light exposure, the pronounced change in dose/response curve was observed at 25 mW/cm^2 yielding a total dose of 360 J/cm^2 [181]. The peculiarity of Lawwill`s study was evaluation of light damaging effects using three independent methods namely electroretinography (ERG) (each second day after the exposure), ophtalmoscopy (daily after the exposure) and histology (1-4 month after the exposure). While ophtalmoscopy did not show any obvious correlations, the ERG data fitted well to histopathologic changes revealing the above-mentioned thresholds. It should be noted that histologically the light-induced damage began with edema and disruption of outer segments of photoreceptors and only afterwards results in involvement of PRE indicating that the model exhibited some properties of class I (Noell) damage as well. These observations were confirmed by Lawwill *et al*. in the further studies revealing differences in light damage response in rabbits in comparison to rats and primates. Although retinas of first two species are both rod-dominant, more energy was required in the rabbit, and their action spectrum was different in that the rabbit retina was more sensitive to short-wavelength light than to medium-wavelength, and the pigment epithelium was relatively less affected. In rabbit retina the PD was also observed in nonreceptor cells. Furthermore, light damage in rabbits has many similar features as found in primates which makes rabbit model of PD feasible for biomedical applications [194].

General Approaches to Inducing PD in Rabbits

Based on early works by Lawwill and co-authors as well as later studies, several approaches to inducing PD in rabbits have been developed. The first and most common approach provoking more frequently Ham class damage includes the following conditions [173, 174, 195-201]. The animal is subjected to general anesthesia and its one eye is exposed to the light from directly positioned source, while the fellow eye is kept in the dark as a control. During the exposure, the pupil of the experimental eye is dilated and the eye is permanently moistened with artificial tears. The light is provided by either broad spectrum source (such as xenon arc or halogen lamp) or the source of defined wavelength (xenon arc lamp equipped with optical light filters) or monochromatic laser lines. A homogeneous exposure on the rabbit retina is sometimes achieved using special optical system permitting direct observation of the light projection on the retina. Using this configuration the rabbit eye can be subjected to the light of defined irradiances calculated in watts per square centimeter of the exposed surface. The exposure to the light of different wavelengths with irradiances ranging from ~ 1 to 300 mW/cm^2 normally continues for 0.5-5 hours yielding cumulative doses of ~ 3-5000 J/cm^2. The light damaging effects are quantified using ERG, fundoscopic and/or histological studies using light and/or electron microscopy. This battery of tests can be expanded by including fluorophotometry (to evaluate blood-retinal barrier dysfunction by measuring fluorescein leakage) [201], immunohistochemical detection of apoptosis markers in retinal cells, evaluation of superoxide *in situ* [173], detection of lipid and protein oxidation in retinal homogenates/extracts [173, 174]. Although this approach does not fairly simulate the conditions of natural light exposures, it can be easily standardized and used for various comparative studies. The second approach is based on the procedure developed by Hansson in 1970 for rat PD models [202]. In this approach the unrestrained animals are exposed to broad-spectrum constant or intermittent light of different intensities in cages with or without mirror interiors [203-205]. Prior to the experiment, the rabbits are dark adapted and in some cases their pupils are dilated. The light damaging effects are assessed using ERG or histological studies. This approach better reflects the natural light conditions. Nevertheless, its major weaknesses is the ability of the animal to freely move which makes impossible calculating the dose of irradiation or at least keeping it constant within the series of experiments. This model is not comprehensively characterized which hinders its attribution to one of the damage classes. Finally, an approach was developed for long-term experiments aimed at establishing of effect of retinal degeneration induced by violet and blue light on the levels of expression of defined retinal proteins [175]. In this model the rabbits` eyes are exposed for 2 years to circadian cycles of white light, blue light and white light lacking short wavelengths. The feature of this approach is application of blue-light filtering intraocular lenses that were implanted into the eyes of some of experimental animals

whereby the efficacy of prevention of phototoxic effect on the retina was analyzed.

Characterization of PD to the Retina in Rabbit Models

The first approach was utilized in a number of works aimed at characterization of retinal damage in rabbits at cellular level depending on intensity, duration and spectral composition of the damaging light as well as corresponding morphological and biochemical features [173, 174, 195-198]. In some cases the focus was made on pathological changes in RPE cells representing primary targets of the Ham damage [199-201]. It should be noted that severity of the PD induced in these studies not always correlate with irradiance dosing suggesting that the latter still requires general standardization [181].

In series of works by McKechnie and co-authors, the rabbits` retinae were exposed for 1 hour to broad spectrum light (400-1,150 nm) with irradiances ranged from 40 to 390 mW/cm^2 and the sings of retinal damage were analyzed by means of light and electron microscopy immediately after the exposure or after various periods (up to 4 weeks) allowing cells to recover from the injury. They demonstrated that the exposure with irradiance of >310 mW/cm^2 affected the outer segments of both the rod and cone cells with cone outer segments were more susceptible to photic damage [195]. Further it was indicated that degree of PD is variable even within the same eye and its distribution is irregular suggesting that several damaging mechanisms are involved [197]. Finally, they found that PD produced by defined irradiances of 140 (‘low’) or 230 (‘high’) mW/cm^2 differ not only in extent of injury but also in potency for further recovery [196]. The effects of exposure to the ‘low’ irradiance included fragmentation of photoreceptor OS, edema of ONL and distention of RPE cells, all of which however were almost completely recovered in 4 weeks. By contrast, the exposure to the high irradiance resulted in complete destruction of photoreceptors and RPE in the center of the lesion, similarly to that described after photocoagulation of the rabbit retina [206]. The damage was irreversible and resulted in substitution of photoreceptors and RPE cells by regions of gliosis [196].

In the study by Hoppeler and co-authors the time-course of Ham damage in rabbits was monitored by their exposure for 5-30 minutes to the 400-550 nm visible light with irradiance of 25 and 46 mW/cm^2. The histological analysis revealed RPE swelling after 5 min and photoreceptor OS disruptions associated with vesiculation of disk membranes at the base of OS after 20 min of exposure. The more prolonged illumination resulted in degeneration of outer nuclear layer (25 min) and edema of nerve fiber layer (30 min). With the course of time these damages become supplemented by macrophage invasion removing apoptotic photoreceptor cells (6-12 days), PRE proliferation (1-6 weeks) and complete loss of photoreceptors (8 weeks). Notably, comparison of these morphological changes in pigmented and albino rabbits revealed no effect of pigment epithelial melanin on the severity of the PD [198].

The other series of works focused on characterization of Ham class PD to PRE cells after blue light-induced injury [199-201]. The PD was produced by exposure of pigmented/albino rabbits for 0.5-5 hours to broad-band (400-520 nm) of narrow-band (408, 418, 439, 485 and 500 nm) blue light with irradiances of 1.7-50 mW/cm^2, or to broad-band yellow light (510–740 nm) with irradiance of 280 mW/cm^2. The PRE cells of the exposed retinae were characterized functionally (evaluating the blood-retinal barrier at the RPE *via* measuring the leakage of injected fluorescein by fluorophotometry) and morphologically (by light and electron microscopy). It was found that RPE structural and functional damage is most pronounced after exposure to 418 nm light. The threshold doses for RPE damage are 1600, 50 and 18 J/cm^2 for broad-band yellow light, broad-band blue light and 418 nm blue light, respectively. In addition, it was demonstrated that the presence of melanin in PRE (pigmented *vs* albino rabbits) neither required nor protect from blue-light-induced PD to these cells thereby confirming the results by Hoppeler *et al*. [198].

Recently, Saenz-de-Viteri and co-authors created a rabbit model of PD by illuminating the animals for 2 hours with white light form a 150 W fiber optic halogen lamp equipped with optical fiber sources at distance of 0.5–1 cm from the cornea [173]. Morphologically, the main light-induced effects were thickening of neurosensory retina and outer nuclear layer as well as vacuolization in OS, more pronounced a 1 week after exposure. Such moderate PD may be presumably classified as Noell damage. Comparing to previous studies, this model was characterized more comprehensively by additional evaluating of immediate and late post-injury (after 1 week) biochemical changes in the retina. Thus, biochemical studies immediately after the exposure revealed sings of apoptosis and oxidative stress in the retina expressed in caspase-1 and caspase-3 activity (detected in INL) and increase in lipid peroxidation (detected in retinal homogenetes) and superoxide production (detected *in situ* in outer nuclear layer, inner nuclear layer, and ganglion cell layer), respectively. Both processes are likely decelerated after 1 week post-exposure although caspase-1 remains active which together with macrophage infiltration in the retina [196, 198] indicates development of long-term inflammatory response. Nevertheless, the data obtained using this rabbit model strongly suggests that PD is initiated by light-induced oxidative stress which within the photoreceptors might result in oxidation of cellular components such as visual cycle proteins and thereby initiate apoptosis.

Indeed, in the study by Zernii and co-authors the light-induced oxidation of distinct photoreceptor proteins was demonstrated using similarly designed rabbit model of PD [174]. The rabbits` retinae were illuminated with 100 W halogen lamp at distance of 30 cm yielding even lower irradiance (~ 1.1 mW/cm^2) comparing to the model by Saenz-de-Viteri *et al*. [173]. However, in this case the exposition was prolonged up to 5 hours (1, 2 and 5 hours) thereby increasing total dose of irradiation to ~ 20 J/cm^2. Consistently, histological examination revealed that illumination for 1-2 hours had no significant effect on the structure of the retina, while 5-hour exposure induced multifocal retinal damage manifested as disarrangement of photoreceptor layer including swelling and shortening of OS associated with migration of RPE cells, retinal edema and even minor granulocytic infiltration. The OS debris was accumulated between RPE and the retina, giving rise to

retinal buckling and detachment. The light-induced changes in this rabbit model were further characterized on protein level focusing on recoverin and arrestin, that were previously suggested to be the redox-sensitive proteins [207-209]. Western blotting and mass spectrometry analysis of retinal extracts reveal illumination time-dependent accumulation of oxidized forms of both proteins such as their disulfide homodimers. This property of recoverin together with redox dependence of its thiol-disulfide equilibrium determined *in vitro* allowed estimating alterations in redox potential in rod outer segments associated with light-induced oxidative stress. Additionally, in the case or recoverin, the formation of its higher order disulfide cross-linked species, including a fraction of mixed disulfides with intracellular proteins such as tubulin and proteosome subunits, was observed. It was suggested that increased propensity to multimerization/aggregation and covalent binding to cellular proteins deteriorates function of oxidized recoverin, which together with arrestin oxidation may affect rhodopsin desensitization and consequently cause apoptosis of photoreceptors. The rabbit model developed in this study might be useful for the further characterization of light-induced oxidation of retinal proteins thereby unraveling molecular mechanisms of PD to the retina.

Biomedical Applications of Rabbit Models of PD

The division of PD in two classes is necessary for standardization of the damage administrating procedures for comparative research studies. Yet, in order to support biomedical objectives both classes of PD might be employed since the actual damaging conditions may vary in light wavelengths and intensities and may simultaneously affect photoreceptors, RPE and other retinal cells. Rabbits are well suited for these purposes since they are likely capable of developing both classes of light-induced damage to the retina (see hereinbefore). In general, the majority of the above mentioned rabbit models is relevant for evaluation of a number of parameters of PD to the retina on cellular, biochemical and molecular levels especially if up-to-date techniques are used. Thus, their biomedical applications may include mechanistic investigations of light-induced retinopathies and various light-affected ophtalmological disorders such as AMD [174]. Furthermore, these models are feasible for monitoring of light-induced complications of visual disorders with non-photogenic etiology i.e. diabetic retinopathy [210]. Another important direction of employing rabbit models of PD includes characterizing light-induced iatrogenic injuries during ophthalmological examinations and ocular surgery interventions such as keratoprosthesis, vitrectomy, corneal refractive procedures and cataract surgery (complications associated with pseudophakia) [176, 211]. Finally, rabbit models are suited for studies of the exacerbating impacts of dietary and prescription of pharmaceuticals on susceptibility to PD.

Understanding the mechanisms of PD provides a way to developing non-invasive and invasive approaches for prevention of the injury. In this context, rabbit models are appropriate for trialing medicinal treatment due to their feasibility for monitoring drug efficacy, safety, pharmacokinetics and pharmacodynamics. Since oxidative stress is one of the major damaging factors in PD, pre-treatment with various antioxidants was suggested as efficient approach against phototoxicity [178]. Indeed, pre-treatment with antioxidants reduced the severity of PD in rats [212]. Consistently, the protective effect on the retina according to the ERG studies was observed in rabbit model of PD in the case of nutrient-derived antioxidants anthocyanins [204, 205]. Furthermore, rabbits are well suited for ocular surgery which makes rabbit models of PD feasible for trialing anti-phototoxic surgical approaches such as implantation of UV-protective IOLs. It is known that the natural eye lens absorbs UV irradiation, while cataract extraction/lens replacement increases susceptibility of the retina to UV light in aphakic and pseudophakic eyes [213]. The modern approach for reducing this damaging effect is employment of IOLs maintaining filters that block UV transmission. The rabbit models of PD were used to analyze efficacy of prevention of phototoxicity on the retina by using such blue-light filtering IOLs [175]. Recently developed new class of IOLs contains photosensitive silicone macromers which allows adjusting correct refraction of the lens after implantation by low-intensity beam of near-UV light. In this case rabbit model was successfully applied for evaluating retinal susceptibility to UV irradiation delivered by an IOL-adjusting optical system [211].

Overall, the employment of rabbit models of PD in ophthalmological studies is quite limited to the date. However, recently developed approaches for pharmaceutical treatment of PD-associated visual diseases as well as modern techniques in ocular surgery impart new relevance to using rabbits in studies of retinal phototoxicity especially considering their high accessibility, easy handling and unique features of the rabbit eye.

CATARACT

Cataract is an opacification of the intraocular crystalline lens, which is caused predominantly by age-related changes and affected by many other factors. Cataract manifests as a visual impairment and in some cases it may lead to eventual blindness [214]. There are three most common types of age-related cataracts including (i) nuclear cataract (hardening and yellowing of the central part of the lens), (ii) cortical cataract (discrete opacities in the cortex of the lens), and (iii) posterior subcapsular cataract (opacification within the posterior cortical layer just under the posterior capsule) [215]. Other types of cataract may be either congenital or secondary i.e. developed as a result of trauma or in response to iatrogenic procedures such as an ocular surgery [215]. Development of age-related cataract is a complex process which is exacerbated by UV exposure, smoking, diabetes and steroids administration [215]. Mechanisms of cataractogenesis involve nuclear sclerosis of the lens, oxidative damage of lens epithelial cells, chemical and structural alterations in the major lens proteins crystallins, pigmentation of crystallins, as well as changes in the lens ionic homeostasis (i.e. magnesium deficiency) [215-217].

To date, no effective medicinal approaches to prevent the development or progression of cataract have been suggested. The only accepted therapy of the disease is surgical replacement the affected lens with artificial intraocular lens (IOL). Although the success rate of such surgeries exceeds 97%, they are at risk of both immediate and deferred side effects [218-220]. Therefore, development of effective

agents for treatment of cataract is still extremely important and represents a major goal for many research groups and pharmaceutical companies [221]. Such projects would be impossible without adequate animal models of the cataract, which are necessary for extrapolation of the results from animals to humans.

Rabbit Models of Cataract

Rabbits are commonly used in studies of cataractogenesis mechanisms and revealing of the impacts of various risk factors on cataract formation. Rabbit models are also employed for preclinical safety evaluation of new cataract surgery and laser vision-correction techniques, studies of posterior capsule opacification as a complication of cataract surgery or even photochromic stability studies of new IOLs [214, 222-224]. Several physical and biochemical approaches for induction of cataract in rabbits were developed.

Experimental Cataract Induced by Physical Impacts

It was shown that various physical impacts such as an electric current or microwave and UVA radiation can be used for the induction of pathological changes in rabbit lens, which may be related to the formation of human cataract. The first report on electricity-induced cataract comes from the first quarter of the XVIII century, when St. Ives documented it in 1722 [225]. Currently, it is known that electric cataract can be produced by the passage of 28 to 150 watt-seconds of electrical energy through the rabbit eye using the electrodes placed directly on the proptosed globe [226]. Yet, a number of studies using rabbits showed that therapeutic amounts of electricity can be used safely around the globe without inducement of cataract [225].

Broad expansion of modern technological devices like radio, TV, cell phones transmitters and receivers, radars and video displays emit microwaves exhibiting cataractogenic potential [227]. The studies of the ocular impacts of microwave irradiation often involve rabbits as model animals. Moreover, the above-mentioned phenomena can be utilized for development of reproducible models of cataract in rabbits. Thus, it was found that cataract can be induced by exposure of rabbit eye to 2450 MHz microwaves for more than 30 min yielding relatively high irradiation doses (≥150 W/kg) and temperatures (≥41°C) [227, 228]. Similarly, reproducible development of cataracts is observed after exposure of rabbits to UVA irradiation for 1 h using monochromatic 365 nm light [229].

Experimental Cataract Induced by Biochemical Factors

Besides physical impacts, cataract can be induced by application of various biochemical factors involved in cataractogenesis. Thus, cataract develops after feeding of rabbits with synthetic tryptophane-free diet for 30 days [230]. Posterior subcapsular cataract develops in rabbit eyes three months after intravitreal injection of concanavalin A (100 μg in one eye), which causes ocular inflammation [231]. Single intravitreal injection of dispase endopeptidase (0.045 to 0.065 units) also results in a reproducible induction of cataract combined with proliferative vitreoretinopathy and lens luxation [232]. Cataract can be observed within 1-3 days after an intravitreal injection of pro-oxidant diquat (300 nmole) into the eyes of pigmented rabbits confirming involvement of oxygen free radicals in the cataractogenesis. In this case, complete opacity of the lens develops 4-6 weeks after the injection [233]. An additional factor increasing risk of cataract development is diabetes [215]. Indeed, it was found that alloxan-induced diabetes in white rabbits (100 mg/kg alloxan administration) causes cataract formation and lens damage [234, 235].

In general, despite the significant success in cataract treatment using surgical strategies, the problem of the disease is not finally solved. In order to avoid risks and discomfort associated with surgical intervention, alternative medical treatment of the cataracts using effective pharmaceuticals ought to be developed. For these purposes, adequate animal models might be used on the stage of preclinical studies as templates for therapy, and the existing rabbit cataract models provide indispensable and powerful tools for these studies.

UVEITIS

Uveitis is a general term for a group of syndromes characterized by introocular inflammation arising in some cases without any known infectious trigger and often associated with immunological responses to unique retinal proteins [236]. Uveitis is a common cause of vision loss: according to recent estimations it is responsible for 10-20% of all cases of blindness worldwide [237]. Ocular inflammation may appear as a complication of bacterial and viral infections, chemical and metabolic injuries [238]. Elevated risk of uveitis is also innate to many chronic autoimmune diseases, including Behcet's disease, systemic lupus erythematosus, relapsing polychondritis, Wegener`s granulomatosis, and ankylosing spondylitis [239]. The inflammatory processes affect the uvea (choroid, ciliary body, and iris) and the retina. From the anatomical point of view, uveitis can be classified as anterior (iritis, iridocyclitis, anterior cyclitis), intermediate (pars planitis), posterior (focal, multifocal or diffuse choroiditis, chorioretinitis, retinochoroiditis) or panuveitis [236]. Anterior uveitis is characterized by inflammation of the iris and the ciliary body, intermediate uveitis involves the vitreous cavity or the pars plana while posterior uveitis represents inflammation of the retina and the choroid. In the case of panuveitis all layers of the uvea (both the anterior and posterior parts of the eye) are affected [236].

In most clinical cases it does not seem possible to fully understand etiology of uveitis. Meanwhile, accumulated data evidence that intraocular inflammation associated with the disease is accompanied by the breakdown of the blood-aqueous barrier and consequent cellular infiltration, increase in protein permeability and upregulation of cytokines, chemokines and adhesion molecules in aqueous humor and uveal regions. The exposure of the ocular tissues near the blood-aqueous barrier to inflammatory cytokines and chemokines can cause cytotoxicity ultimately leading to blindness. The increased knowledge in basic inflammatory mechanisms and the progress in pharmacology have improved treatment strategies for ocular inflammation. The current mainstay of uveitis treatment is immunosuppressive therapy, especially by corticosteroids, which cause anti-

inflammatory effects and down-regulate cytokines production. However, these approaches induce temporary side effects and are not very selective. Moreover, topical steroids are often unable to control inflammation due to their poor ocular penetration [240]. Thus, despite considerable success in understanding of pathogenesis of uveitis, a search for effective and safe disease-specific interventions is still required [241].

Experimental Uveitis Studies Using Animal Models

Considering ethical issues and other limitations affecting human studies, animal models of uveitis represent a feasible tool for understanding of basic mechanisms of the disease and evaluation of its prospective therapies. Since none of the animal models can solely reproduce the full complexity of uveitis, it is important to develop and implement a variety of models mimicking different clinical and immunological manifestations of the human disease. During last decades, several experimental animal models of uveitis have been created using mice, rats and rabbits [20, 242, 243]. Currently, mice and rats are in high-demand for studying basic immunological aspect of uveitis, largely owing to availability of the corresponding well-characterized genetic models. However, rather small eye size restricts using rodents in pharmacological studies. Although the absence of well-characterized inbred strains makes rabbits less appropriate for studies of immunological mechanisms of uveitis, this species is well suited for development of corresponding therapeutic approaches including trialing of new drugs and drug delivery systems. The size of rabbit eye is close to human one which makes rabbits appropriate for studies of kinetics of intraocular penetration, absorption and elimination of various human drugs [244]. The rabbit models, which were most frequently used for evaluation of novel therapies of uveitis are described in the next section.

Rabbit Models of Uveitis

Endotoxin-Induced Uveitis (EIU)

Endotoxin-induced uveitis (EIU) is the most common established animal model of intraocular inflammation. The pathologic process is triggered by intraocular injection of lipopolysaccharide (LPS), which is a component of cell wall of gram-negative bacteria [245]. LPS induces inflammation through activation of Toll-like receptor 4, a member of the family of pattern-recognition receptors of the innate immune system. This receptor initiates intracellular signaling pathway thereby activating nuclear factor NF-kB and mitogen-activated protein kinases to induce expression of various proinflammatory cytokines and chemokines (IL-6 [246, 247], TNFα [248], MCP1 (monocyte chemoattractant protein 1) [249]) and production of nitric oxide. All these mediators contribute to breakdown of the blood-ocular barrier and infiltration of leukocytes and proteins in aqueous humor, resulting in development of EIU [245]. The advantages of this model are easy induction, good reproducibility and high speed of disease progression (maximal inflammatory reaction occurs within 24 hours) [245].

Intravitreal injection of 1-100 μg LPS was found to be optimal for induction of EIU in rabbits. During the first 3 hours after the injection epiphora, blepharospasm, ciliary congestion, and iris hemangiectasia are developed in experimental eyes. Six hours post-injection aqueous flare and leukocytes can be observed, while after 12 hours the more severe inflammation characterized by miosis, iris opisthosynechia, occlusion pupillae, flocculation exudation, and vitreous opacity is appeared. The uveitic scores can be normally evaluated after 24 hours since LPS injection [250]. The LPS-induced inflammation in rabbits is generally considered to be anterior uveitis. Consistently histological studies demonstrated leukocyte infiltration mainly in ciliary body and iris. Inside the rabbit eye, LPS up-regulates expression of IL-6 in cornea and IL-1β in iris [251]. The aqueous humor becomes enriched with other inflammatory protein mediators (TNFα, IL-1β, IL-8 and MCP-1) as well as released leukocytes and other inflammatory cells [249, 252]. The amount of leukocytes in the aqueous humor gradually increases and peaks at 24 hours after LPS injection. Aqueous protein concentration also gradually increases, reaches maximum at 24 hours, and then decreases in the course of 2-3 days [252].

The data presented allow to conclude that EIU in rabbits represents an important animal model for studying acute anterior uveitis in humans [253]. Indeed, this type of the disease accounts for 75–90% of total cases of human uveitis [236]. The model is successfully used to test efficacy of existing immunosuppressive agents and anti-inflammatory drugs and can be employed for development of new therapeutic approaches for treating acute anterior uveitis [254-256].

Experimental Autoimmune Uveitis (EAU)

A typical method for induction of experimental autoimmune uveitis (EAU) in rabbits is their immunization with retinal antigens. Various retinal proteins (i.e. rhodopsin, recoverin, phosducin) may be employed for this purpose although the most frequently used uveitogenic agents are visual arrestin (or S-antigen) and interphotoreceptor retinoid-binding protein (IRBP) [20, 257-259]. Both proteins are involved in visual reception/transduction and in normal retina are localized in photoreceptor cells area. Arrestin is expressed specifically in photoreceptors where it participates in regulation of phototransduction whereas IRBP is found in the interphotoreceptor matrix (between the retinal pigment epithelium and the neurosensory retina) and plays a role in retinoid cycle. Consistently, immunization of rabbits by arrestin or IRBP results in autoimmune inflammation with lymphoid infiltration predominantly in posterior choroid and causes subsequent photoreceptor degeneration [258, 259]. The antigens can be delivered in rabbits *via* subconjunctival (arrestin, 50 μg [259]) or subcutaneous (arrestin, 800 μg [257]) or intramuscular (IRBP, 150 μg [258]) injections of the protein mixed with complete Freund's adjuvant. For instance, 2-3 weeks after subconjunctival injection of arrestin, IgG and macrophages infiltrate RPE, neurosensory retina and choroidal interstitium suggesting breakdown of the outer blood-retinal barrier. From fourth till seventh weeks the retinal pathology becomes widespread and

Table 2. Rabbit models of ocular diseases.

Disease	Form	Induction	Manifestations	Reference
Dry Eye Syndrome		Surgical removal of the greater superficial petrosal nerve	Decreased tear production, breakdown of the protective tear film, increased frequency of blinking, dry spots on the mucosal surface of the eye, reduced number of goblet cells	[50, 51]
		Topical administration of 1% atropine sulfate	Decreased tear production, dry spots on corneal surface	[57, 58]
		Instillations of benzalkonium chloride	Eye redness, inflammatory cell infiltrations, decrease in goblet cell density, ultrastructural disorganization of cornea and conjunctiva, mucin deficiency	[60-62]
		Injection of stimulated lymphocytes into the lacrimal gland	Decreased tear production, corneal damage, histologically verified autoimmune dacryoiditis	[65, 66]
		Direct evaporation of tears (open-fixed eye)	Dry spots on corneal surface, epithelial thinning	[67, 68]
Glaucoma	Primary open angle	Subconjuctival injection of steroids or adrenalin	IOP elevation, abnormal accumulations of mucopolysaccharides in the the anterior eye chamber, 50% reduction of the optic nerve disc diameter	[92-96]
		Injection of α-chymotrypsin into the posterior chamber	Buphtalmia, stable IOP increase, retinal degeneration and excavation of the optic nerve disc	[97-99]
		Injection of viscoelastics (HPMC) into the anterior chamber	Optic nerve cupping, ganglion cell loss, retinal detachment, buphtalmia, lens swelling, thinning of the inner limiting membrane between the retina and the vitreous body	[102]
	Primary angle closure	Water-loading	Acute IOP increase	[103-105]
		Ablation of the trabecular meshwork with argon laser	Stable ocular hypertension, buphtalmia, optic nerve cupping	[106, 107]
	Primary congenital	Spontaneous onset in New Zealand White rabbits	Disorganization of the trabecular meshwork, reduced outflow facility, chronic ocular hypertension, buphtalmia, optic nerve degeneration	[109-113]
	Normal tension	Intravitreous injection of endothelin-1	Reduction of blood stream velocity, occlusion of retinal arteries, axonal loss, demyelination of the intraocular region of the optic nerve, gliosis, hypertrophy of connective tissue	[118-122]
Age-Related Macular Degeneration	Early-onset (dry)	Vitreous autotransplantation	Drusen-like changes	[139]
		Subretinal implantation of lipofuscin mimicking particles	Drusen-like changes, sub-RPE choroidal neovascularization, abnormal fundus autofluorescence	[140, 141]
		Cholesterol-enriched diet	Changes in the RPE cells, thickening of the Bruch's membrane	[142]
			Drusen-like changes, increased level of β-amyloid, increased generation of reactive oxygen species	[143]
	Choroidal neovascularization	Laser photocoagulation	Microscopic neovascularizations	[144, 145]
		Subretinal injections of linoleic acid hydroperoxide	Histologically detected choroidal neovascularization, damage of the outer retinal layers	[146, 147]
		Subretinal implanting of bFGF-impregnated gelatin microspheres	Choroidal neovascularization, angiographic leakage	[149, 150]

(Table 2) contd…..

Disease	Form	Induction	Manifestations	Reference
		Subretinal injection of bFGF/lipopolysaccharide in Heparin-sepharose beads	Neovascularization in the subretinal space, Bruch's membrane injury, neovascularization in the sub-RPE space, angiographic leakage	[151]
		Subretinal injection of VEGF mixed with matrigel	Subretinal neovascularization, angiographic leakage	[152]
		Transfer of VEGF gene using adenoviral vector (intravitreal or subretinal injection)	Subretinal neovascularization, macrophage stimulation, retinal edema, loss of photoreceptors	[154]
		Intravitreal implantation of VEGF/bFGF-containing pellets	Subretinal neovascularization, hemorrhages and retinal detachment	[155]
	Subretinal hemorrhage	Subretinal injection of autologous blood	Subretinal hemorrhage, damages of photoreceptors and irreversible retinal destruction	[157]
		Subretinal injection of balanced salt solution, choroid puncture	Moderate subretinal hemorrhage lesions	[159]
		Sub-RPE injection of autologous blood	Diffuse sub-RPE hemorrhage	[156]
Light-Induced Retinal Damage		Direct exposure of experimental eye of anesthetized animal to different doses of broad spectrum or monochromatic light		
		Broad spectrum light, 4 h, 15-160 mW/cm^2 or monochromatic 514.5 mn laser, 4 h, 17-47 mW/cm^2	Edema and disruption of photoreceptor outer segments with further damage of PRE	[193]
		Broad spectrum light (400-1,150 nm), 1 h, 40 – 390 mW/cm^2	Edema of outer nuclear layer, fragmentation of photoreceptor outer segments, distention of RPE cells with further destruction of photoreceptors and RPE	[195-197]
		Visible light (400-550 nm), 5-30 min, 25 or 46 mW/cm^2	RPE swelling (5 min), disruption of photoreceptor outer segments (20 min), degeneration of outer nuclear layer (25 min), edema of nerve fiber layer (30 min), macrophage invasion (6-12 days), PRE proliferation (1-6 weeks), complete loss of photoreceptors (8 weeks)	[198]
		Visible light (400-520 nm) or narrow-band blue light (408, 418, 439, 485 and 500 nm), 0.5-5 h, 1.7-50 mW/cm^2 or broad-band yellow light (510–740 nm), 0.5-5 h, 280 mW/cm^2	RPE structural and functional damage most pronounced after exposure to 418 nm light	[199-201]
		White light, 2 h, 150 W lamp at distance of 0.5–1 cm from the cornea	Thickening of neurosensory retina and outer nuclear layer, vacuolization in photoreceptor outer segments, caspase-1 and caspase-3 activity (in inner nuclear layer), increase in lipid peroxidation and superoxide production (in outer nuclear layer, inner nuclear layer, and ganglion cell layer) with further macrophage infiltration	[173]
		White light, 2-5 h, 100 W lamp at distance of 30 cm from the cornea	No damage (2 h); disarrangement of photoreceptor layer including swelling and shortening of photoreceptor outer segments, migration of RPE cells, edema, minor granulocytic infiltration, retinal buckling and detachment (5 h); oxidation of recoverin and arrestin (2-5 h)	[174]
Cataract	Not specified	The passage of electrical energythrough the eye	Lens opacities, vacuoles, posterior subcapsular iridescence	[226]
	Not specified	Microwave irradiation (30 min, ≥41°C)	Lens opacities	[227, 228]
	Nuclear	UVA irradiation	Lens yellowing, losses of NADH and NAD$^+$ in the lens nucleus	[229]
	Not specified	Synthetic tryptophane-free diet, 30 days	Microscopically verified cataract without visiblefundus reflex	[230]
	Posterior subcapsular	Concanavalin A injection	Lens opacities, histologically detectedloss of normalarchitecture of the posterior cortex	[231]

(Table 2) contd…..

Disease	Form	Induction	Manifestations	Reference
	Not specified	Single intravitreal injection of dispase endopeptidase	Graded lens opacities, luxated lenses	[232]
	Not specified	Single intravitreal injection of diquat	Microscopically verified cataract (after 1-3 days), complete opacity of the lens (after 4-6 weeks)	[233]
	Diabetic	Alloxan-induced diabetes	Progression of lens opacities	[234, 235]
Uveitis	Anterior	Intravitreal injection of endotoxin	Breakdown of the blood-ocular barrier, inflammation withleukocyte infiltration and protein accumulation in the anterior chamber	[250-252]
	Posterior (autoimmune)	Subconjunctival/subcutaneous/intramuscular injection of retinal antigens	The breakdown of the blood-ocular barrier, inflammation with lymphoid infiltration predominantly in posterior choroid, degeneration of photoreceptor outer segments in the entire retina with piecemeal total loss of outer segments and loss of some photoreceptor-cell bodies.	[257-259]

includes degeneration of photoreceptor outer segments in the entire retina with piecemeal total loss of outer segments and loss of some photoreceptor-cell bodies [259].

EAU represents a manageable animal model for posterior uveitis in humans [260]. However, currently it is not widely used, mainly owing to the presence of alternative more simple rodent models which are more appropriate for studies of immunological mechanisms of uveitis [20]. Nevertheless, recent progress in ocular pharmacology revealed new applications for rabbit EAU and EIU models. These can be used to explore new drugs and therapies, such as sustained-release delivery of anti-inflammatory agents *via* intraocular implants for treatment of uveitis [22].

CONCLUSION

Rabbit is an important model for clinical, morphological and mechanistic studies of common ocular diseases, including DES, glaucoma, AMD, light-induced retinopathies, cataract and uveitis. Furthermore, rabbit models are successfully utilized for studying various aspects of other more rare ophthalmological disorders (diabetic retinopathy [261], retinal detachment and proliferative vitreoretinopathy [262, 263], ocular allergy [264], retinoblastoma [265], retinitis pigmentosa [266]), which remained beyond the scope of this review. We highlighted some experimental approaches that efficiently simulate human ocular disease in rabbit (Table **2**). Most of these models are widely used for revealing potential therapeutic targets as well as screening for novel drugs. In contrast to rodent models, rabbits present a prolonged course of the ocular manifestations (which however is still of shorter duration than the majority of clinical cases of the corresponding human diseases that are normally characterized by chronic progression). Despite the progress in ocular pharmacology, the majority of substances for treatment of visual disorders are still used in the form of eye drops [7]. Meanwhile, the main aim of ocular therapeutics is to achieve a pronounced pharmacological response by reaching effective drug concentration in the targeted eye tissue for protracted period of time. A modern approach in ocular pharmacology is controlled release of pharmaceuticals using nanoparticles, ocular inserts, intravitreal injections, intra-ocular lenses and implants, and other systems [7, 22-25]. These allow achieving a release of an active agent in the targeted eye tissue within the therapeutic period for up to several years after the application. Preclinical studies of such therapeutic approaches require chronic and long-living animal models. We can expect that rabbit models of ocular disease are feasible for trialing of these modern techniques, which imparts them new relevance in experimental ophthalmology.

AUTHORSHIP CONTRIBUTIONS

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LIST OF ABBREVIATIONS

AMD = Age-Related Macular Degeneration
bFGF = Basic Fibroblast Growth Factor
CNV = Choroidal Neurovascularization
DES = Dry Eye Syndrome
EAU = Experimental Autoimmune Uveitis
EIU = Endotoxin-Induced Uveitis
IOL = Intraocular Lens
IOP = Intraocular Pressure
IRBP = Interphotoreceptor Retinoid-Binding Protein
LHP = Linoleic Acid Hydroperoxide
LPS = Lipopolysaccharide
NTG = Normal Tension Glaucoma
PACG = Primary Angle Closure Glaucoma
PCG = Primary Congenital Glaucoma
PD = Photochemical Damage
POAG = Primary Open Angle Glaucoma
RAS = Rhodopsin Absorption Spectrum
RGC = Retinal Ganglion Cells
RPE = Retinal Pigment Epithelium

SAS = Short Wavelength Action Spectrum

SH = Subretinal Hemorrhages

SR = Solar Retinitis

VEGF = Vascular Endothelium Factor A

CONFLICT OF INTEREST

The authors confirm that this article content has no conflict of interest.

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