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Concise Review

Characteristics of structures and lesions of the eye in laboratory animals used in toxicity studies

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Abstract: Histopathology of the eye is an essential part of ocular toxicity evaluation. There are structural variations of the eye among several laboratory animals commonly used in toxicity studies, and many cases of ocular lesions in these animals are related to anatomical and physiological characteristics of the eye. Since albino rats have no melanin in the eye, findings of the fundus can be observed clearly by ophthalmoscopy. Retinal atrophy is observed as a hyper-reflective lesion in the fundus and is usually observed as degeneration of the retina in histopathology. Albino rats are sensitive to light, and light-induced retinal degeneration is commonly observed because there is no melanin in the eye. Therefore, it is important to differentiate the causes of retinal degeneration because the lesion occurs spontaneously and is induced by several drugs or by lighting. In dogs, the tapetum lucidum, a multilayered reflective tissue of the choroid, is one of unique structures of the eye. Since tapetal cells contain reflecting crystals in which a high level of zinc has been demonstrated chemically, drug-induced tapetum degeneration is possibly related to zinc chelation. The eye of the monkey has a macula similar to that of humans. The macula consists only of cones with a high density, and light falls directly on the macula that plays an important role in visual acuity. Macular degeneration occurring in monkeys resembles histopathologically that of humans. Hence, the eye of the monkey is a suitable model to investigate macular degeneration and to assess drug-induced macular lesions. (DOI: 10.1293/tox.2015-0037; J Toxicol Pathol 2015; 28: 181–188)

Key words: eye, laboratory animals, lesions, structures

Introduction

The eye is one of the sensory organs in vertebrates and is the most uniquely crafted organ. In humans, vision is the most important function for quality of life (QOL) because approximately 80% of external information is obtained from vision. Visual impairments caused by side effects of drugs or chemicals seriously compromise the human social life. Therefore, risk assessments of the ocular toxicity of chemicals are required in several guidelines of nonclinical toxicity studies[1–4]. In toxicity studies, the eye is the only organ in which the inner portion can be observed ophthalmoscopically[5]. For risk assessment of ocular toxicity, it is important to make a comprehensive diagnosis from the findings of clinical, ophthalmoscopical and histopathological observations[6]. Since structures of the eye are different among several species of animals used generally in toxicity studies, understanding of the structural characteristics of the eye is a key point for detection and assessment of ocular lesions[5]. In comparison with humans, there are some structural differences in the eye of laboratory animals, such as no pigmentation in albino rodents and the presence of the tapetum lucidum in dogs. In contrast, the macula in monkeys is one of the similarities with humans. The eye is composed of a variety of tissues such as the cornea, lens, iris, ciliary body, vitreous, retina, optic nerve and sclera, which originate from the neuroectoderm, surface ectoderm, mesoderm and neural crest[7]. Thus, for successful histopathology of the eye, it is desirable for any ophthalmological lesions to be properly positioned in the histological specimens. Several technical supports, such as proper orientation of the lesion, careful sampling, fixation and processing, for histological specimens are necessary. In light of the information mentioned above, histopathological examination of the eye should be performed carefully on a basis of comprehensive understanding. This review focuses on some structural characteristics (pigmentation in rodents, the tapetum lucidum of the retina and a Y-suture of the lens in dogs and the macula in

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monkeys) and spontaneous and drug-induced ocular lesions related to their structural characteristics in toxicity studies.

Structural Characteristics of the Eye in Laboratory Animals

The eye is one of the special sense organs and is composed of various tissues that cooperate systemically and functionally and contribute to maintenance of vision. There are several differences in ocular dimensions and structures among laboratory animals and humans (Table 1)[8–11], although the basis of the visual systems in these animals is essentially similar. Several structural characteristics of the eye in laboratory animals are described bellow.

Rodents

Commonly, albino rats are used in general toxicity studies with ophthalmological examinations. These rats have no melanin in the body, including the eye. The retinal vasculature of rats is holangiotic (totally vascularized in the whole fundus), and the vessels extend radially from the optic disc[12]. The albino condition has a few advantages in ophthalmological examinations. The retina and the retinal and choroidal vasculatures in albino rats can be observed clearly by ophthalmoscopy because there are no pigments in the fundus (Fig. 1a and 1d). As a result, fundus lesions are detected in detail, resulting in improvement of histopathological detection accuracy. In contrast, eyes of pigmented rats consists of many pigmented tissues such as the posterior epithelium of the iris, the outer epithelium of the ciliary body, the pigmented support tissues of the choroid and the retinal pigment epithelium (RPE) (Fig. 1c and 1f). In addition, melanocytes and melanin-ingested macrophages are scattered in various interstitial tissues of the eye. These pigmented tissues absorb excess light irradiation and play an important role in protection of the retina against light damage because the anterior (cornea and anterior chamber) and medial portions (lens and vitreous) of the eye are transparent to allow for entrance of visual information. The

Table 1. Ocular Dimensions of Laboratory Animals and Humans

Species	Axial length (mm)	Corneal thickness (mm)	Anterior chamber depth (mm)	Lens thickness (mm)	Vitreous chamber depth (mm)
Rat[8]	5.98	0.25	0.87	3.87	1.51
Dog[9]	20.8	0.64	4.29	7.85	10.02
Monkey[10]	17.92	0.55	3.24	2.98	11.3
Human[11]	23.92	0.55	3.05	4.0	16.32

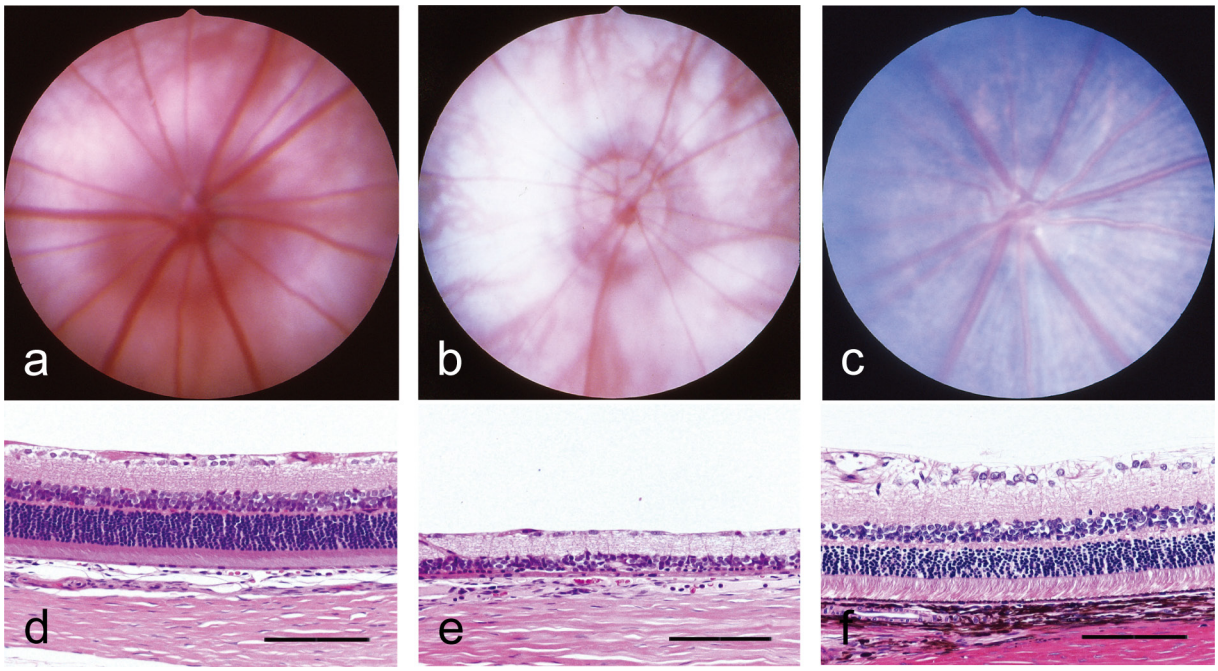

Fig. 1. Fundus and histology of the retina in rats. a) Normal features of the fundus in a 19-week-old male IGS rat. b) Severe retinal atrophy of a 58-week-old male Wistar rat. Note increased fundus reflection, atrophy of retinal vasculatures and pallor discoloration and edematous swelling of the optic disc. c) Normal features of the fundus in a 19-week-old male Zucker rat. Note the dark grayish discoloration of the fundus. d) Normal retinal structures of the same rat as in a). Note that there is no pigment deposition anywhere. e) Severe retinal degeneration of the same rat as in b). Note the loss of the outer retinal layers (photoreceptor layer, outer limiting membrane, outer nuclear layer and outer plexiform layer), disorganization and decreased cell numbers of the inner nuclear layer, decreased thickness of the inner plexiform layer and decreased number and shrinking of the ganglion cells. f) Normal retinal structures of the same rat as in c). Note the pigmentation in the retinal pigment epithelium, the choroid and interstitium of the sclera. HE stain. Bar = 100 µm.

photoreceptors of the retina in the rat are composed of rods (approximately 99%)[13]. The rods function in dim or reduced illumination, and this is related to the fact that the rat is a nocturnal animal. It is well known that the RPE plays several important roles in maintenance of physical homeostasis and visual functions of the retina, especially for photoreceptors[14]. Since many chemicals show significant affinity to melanin and are accumulated in the RPE[15], pigmentation of the eye would likely affect the outcome of toxicity tests.

Dogs

Since the eyes of dogs are relatively large, it is possible to perform detailed observations in the anterior portion, lens, vitreous and fundus in ophthalmological examinations. For histological confirmation of ophthalmoscopic lesions, proper orientation and subsequent appropriate preparation of the ocular specimen are important. The fundus of the dog is divided into two portions, the tapetum and non-tapetum areas (Fig. 2a). The tapetum area is called the tapetum lucidum, which is a layer of reflective tissue in the choroid and is composed of green-, yellow- and blue-colored elements, and is one of the structural characteristics of the eye in dogs (Fig. 2a and 2b). The tapetum lucidum is roughly triangular in shape and is located at the dorsal portion of the fundus when observed in an ophthalmoscopic examination[13]. The non-tapetum area, called as the tapetum nigrum is deep brown in color, owing to pigment in the RPE (Fig. 2a and 2c). The tapetal color varies in association with breed, species, age and amount of pigmentation[13]. Microscopically, the tapetum lucidum is interposed between the branching vessels in the choroid and the single layer of the choriocapillaris beneath the retina[13]. The tapetum lucidum acts to amplify and reflect light back through the photoreceptor layer again in dim light conditions[7]. The RPE cells over the tapetum lucidum are unpigmented normally (Fig. 2b)[13]. The tapetal cells of beagle dogs contain reflecting crystals in which a high level of zinc (as zinc-cysteine complex) has been demonstrated chemically[16]. The retinal vasculature of dogs has a holangiotic pattern, as do those of rats and monkeys and approximately 20 cilioretinal arterioles radiate from the optic disc, with three to four major veins[13]. In a normal lens of dogs, a Y-shaped suture pattern of lens fibers is observed by ophthalmoscopy and is related to the development of the lens (Fig. 2e)[13]. The lens fiber cells, which are wide shaded bands arise from the tip of a branch of the suture and insert into a fork at the posterior pole[7]. Generally speaking, the Y-suture is difficult to detect in a histological specimen, despite it being one of the common sites for the initiation of cataract[7].

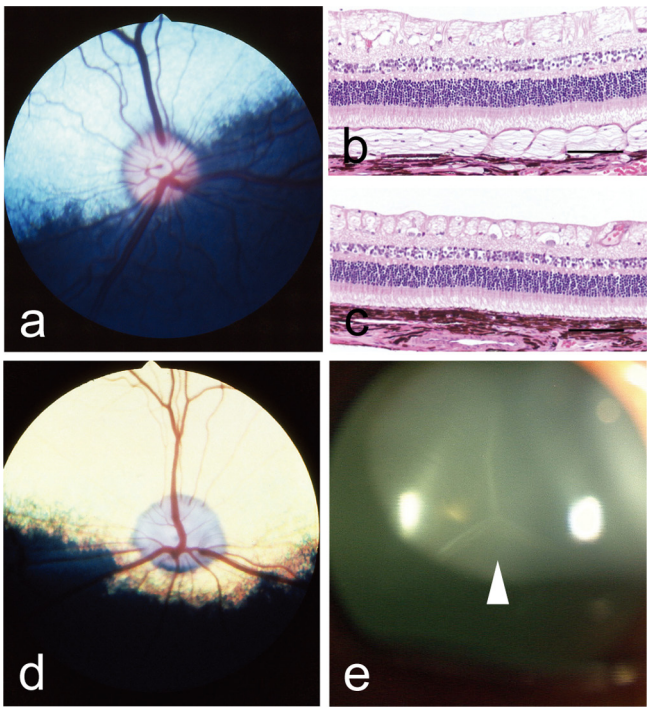

Fig. 2. Fundus, histology of the retina and gross appearance of the lens in beagle dogs. a) Normal features of the fundus in an 8-month-old male dog. Note the bluish-green coloration of the tapetum lucidum in an upper part of the retina. b) Normal retinal structures of the tapetum lucidum of the fundus of the same dog as in a). Note the multilayered tapetum cells between the retinal pigment epithelium (RPE) and the choroid and the decreased pigmentation of the RPE. c) Normal retinal structures of the non-tapetum portion of the retina of the same dog as in a). Note that there are no tapetum cells between the RPE and choroid. HE stain. Bar = 100 µm. d) Drug-induced decoloration of the tapetum lucidum in the retina of a 10-month-old male dog. The tapetum lucidum is changed to a light yellowish color. e) A Y-suture (arrowhead) of the lens in a 22-month-old male dog. The suture is normally observed in a posterior portion of the lens.

Monkeys

In toxicity studies, cynomolgus monkeys (*Macaca fascicularis*) and rhesus monkeys (*Macaca mulatta*) are commonly used. These nonhuman primates are the only laboratory animals that have a retinal structure similar to that of humans, that is, the macula (Fig. 3a). The macula is located at the posterior pole, the temporal side of the optic disc, in the fundus and constitutes the zone of greatest visual acuity[17]. The macula is also characterized by yellow macular pigment, which consists of the plant pigment lutein and zeaxanthin. The center of the macula has multiple features designed to optimize spatial resolution. The macula consists of the cones with a high density and does not include retinal vessels (Fig. 3b)[13]. The cones function in bright light and provide sharp visual acuity and color sensitivity[13]. On the other hand, the rods, which function in dim or reduced illumination, provide detection of shapes and motion[13]. In a central pit of the macula, called the fovea, the overlying retinal layers are greatly reduced, and there are no rods or other retinal cells[17]. The thinning of the retina at the macula serves the intensity of visual acuity because light falls directly on the macula, which contains a large number of cones[17]. Hence, the eyes of monkeys are a suitable model to assess chemical-induced macular lesions and investigate macular degeneration.

Spontaneous and Toxic Ocular Lesions in Laboratory Animals

Rodents

In albino rats, age-related retinal atrophy is one of the common ocular lesions, and the lesion is ophthalmoscopically observed as various hyper reflective changes in the fundus (Fig. 1b)[12, 18–20]. This ophthalmoscopic lesion is usually observed as focal or diffuse degeneration of the retina in histopathological and electron microscopical examinations (Fig. 1e)[19, 21, 22]. Retinal degeneration in albino rats is well known to be induced by several drugs[23–27] and also to occur in relation with age and/or light in the animal housing environment[21, 28–34]. Therefore, it is important to differentiate the cause of retinal degeneration. Albino rats are more sensitive to light than pigmented rats, because there is no melanin in the eyes of albino rats. Exposure to continuous illumination in both albino (Slc:SD) and pigmented (ACN/I) rats results in retinal damage only in albino rats[21]. The protective effect of pigment against phototoxicity is due to both RPE pigmentation and screening by melanin in the anterior and posterior uvea, which limits light fluxes in the retina[34]. Light damage of the retina is a frequent finding in long-term safety studies of albino rodents, and the lesion is dependent on the intensity and exposure duration of the light[21, 32, 34]. It is expected that lower light levels should help reduce the incidence of phototoxic retinopathy without impact on study quality[34]. On the other hand, it is suggested that the retinal function in aged pigmented rats is depressed by the age-related decrease of photoreceptor cells, although the depression is relatively slight compared with that in aged albino rats[35]. Pigmented strains of rats are also used in several toxicity studies. Many compounds show significant affinity to melanin and are accumulated in the pigmented compartments of the eye[14]. RPE is a likely target for systemically administered compounds, since the underlying choroid is highly vascularized[14]. However, several drugs bind to melanin without ocular effects, and other drugs induce retinal effects unrelated to melanin binding, suggesting that binding of drugs to eye melanin is not predictive of ocular toxicity[15]. Therefore, it is necessary to assess carefully whether drug-induced retinopathy is related to the melanin affinity of the drug. In addition, monitoring the background incidences of ocular lesions including retinal degeneration in both albino and pigmented rats[18–20, 35–38] contributes to evaluation of ocular toxicity. Albino rodents are also well known to have other several kinds of spontaneous ocular lesions, including cornea dystrophy (calcium deposition), cataract and retinal fold/dysplasia, but these lesions are not described in detail in this review; they have been described in detail in several other articles[18–20, 36–38].

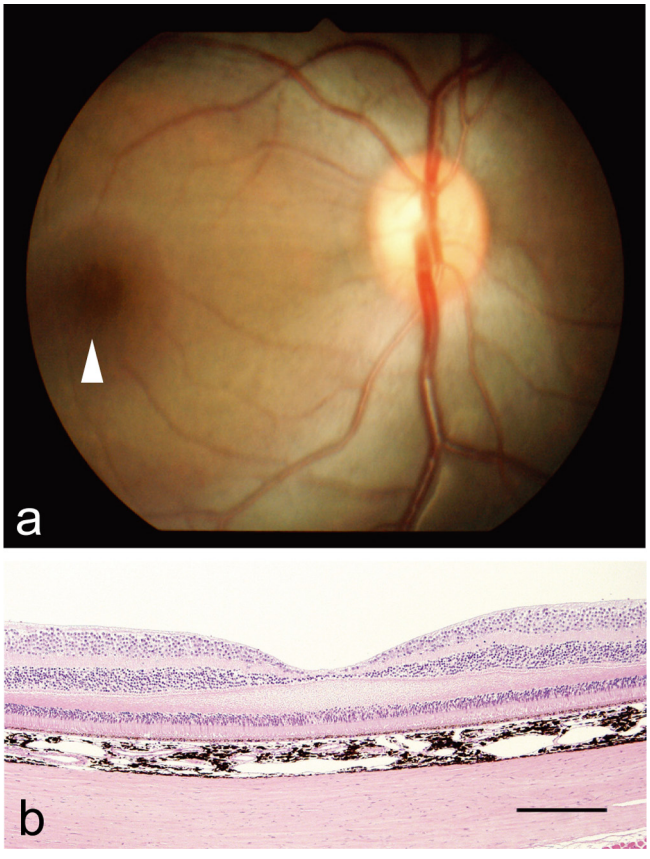

Fig. 3. Fundus and histology of the retina in cynomolgus monkeys. a) Normal features of the fundus in a 6-year-old male monkey. Note the macula (arrowhead) beside the optic disc. b) Normal retinal structures of the macula in the retina of a 4-year-old female monkey. Note the pit formation (fovea) in the retina consisting of cones and a decreased number of other retinal cells. HE stain. Bar = 200 µm.

Dogs

In beagle dogs used in toxicity studies, spontaneous ocular lesions are not frequent compared with those in rats and mice. Several histopathological studies on the background reference data described infrequent spontaneous lesions detected in the eyes of beagle dogs used in toxicity studies[39–45]. The reasons for this seem to be that the breed of dogs used in toxicity studies is limited to the Beagle, the test period is relatively shorter, up to one year, than those in rodents, and the dogs are used at young age in toxicity studies. However, there are a few case reports on spontaneous ocular lesions, such as focal inflammatory cell infiltration in the conjunctiva and disarrangement of the retinal structures[45], corneal ulcer[46], corneal dermoid[47], unilateral ocular subalbinism[48] and choroidal melanoma[49] in beagle dogs. On the other hand, retinal degeneration (tapetum degeneration) has been described in relation with the administration of several drugs in beagle dogs[50–55], called as toxic tapetopathy[55]. Oral administration of zinc pyridinethione (ZPT)[50, 56, 57], a beta-adrenergic blocking agent (SCH 19927)[51], a synthesized chymotrypsin inhibitor (FK-401)[53], an azalide antibiotic (CP-62993)[54] and an antipsychotic agent (1192U90)[55] and intravenous administration of a macrolide antibiotic (rosaramicin)[52] to beagle dogs resulted in an altered tapetal color (Fig. 2d) with degeneration or necrosis of the tapetum lucidum. However, these drugs induced no retinal lesions

in the eyes of animals without a tapetum lucidum, such as atapetal beagle dogs, mice, rats, pigmented or albino guinea pigs, Mongolian gerbils, rhesus monkeys or cynomolgus monkeys[50, 55, 56]. Since the tapetum lucidum of the retina in dogs contains a high level of zinc (as zinc-cysteine complex)[16], several studies described that drug-induced tapetum degeneration, i.e., toxic tapetopathy, in the eyes of dogs would be related to zinc chelation[50, 51, 55]. In addition, oral administration of ethambutol to beagle dogs also induced decoloration of the tapetum lucidum[58, 59], in which marked swelling and disorientation of parallel rods packed in the tapetal cells were observed electron microscopically[58]. Numerous pharmacologic agents and chemicals have been noted to induce cataracts in the dogs used in toxicity studies[60]. Toxic cataracts appear initially in a variety of locations within the lens and are related to alteration of Na,K-ATPase pumps, iron or osmotic balance or cell membrane permeability[60]. Toxic cataracts in dogs often begin either in the anterior and posterior cortical region near the equator or in the Y-suture regions[60]. High doses of various hydroxymethylglutaryl-CoA (HMG-CoA) reductase inhibitors produce anterior and posterior subcapsular cataracts that are seen initially as accentuation of the Y-suture lines[61]. Feeding a 30% galactose diet induced an accentuation of anterior and posterior sutures of the lens in beagle dogs and ultimately resulted in sugar cataract[62].

Monkeys

Age-related macular degeneration (AMD) has become one of the leading causes of blindness in the industrialized world in adult humans older than 65 years[63]. The macula is a structure of the eye unique to humans, apes and monkeys and plays a role as the zone of greatest visual acuity[17]. Therefore, nonhuman primates are a potentially valuable animal model for investigating macular diseases of humans. A variety of spontaneous ocular lesions were reported in cynomolgus monkeys used in toxicity studies[11, 64–67]. Lesions of the fundus have been reported in a study providing a representative overview of more than 2000 monkeys having a 7.9% incidence of ocular lesions and 6.6% incidence of fundus lesions[11]. Only a few cases of spontaneous macular degeneration, called drusenoid maculopathy, have been described in rhesus monkeys[68] and cynomolgus monkeys[69, 70]. On the other hand, there are a few colonies of monkeys with familial macular degeneration resembling human AMD[71–73]. Drusenoid maculopathy was observed in rhesus monkeys with an incidence of 61%, and prevalence and severity of the maculopathy increased with age[73]. In the fundus of rhesus monkeys with drusenoid maculopathy, many roundish, white drusen are concentrated in and around the fovea[73]. Drusen are focal or diffuse basement membrane products and are produced by the RPE admixed with other materials[74]. Those materials may be trapped within drusen when they pass through from RPE to choriocapillaris[74]. The presence of drusen is known as the earliest sign of human AMD[74]. Histopathologically, drusenoid maculopathy in monkeys is characterized by drusen formation as extracellular deposits between the RPE and Bruch's membrane, detachment of the RPE directly under the fovea and disorganization of the outer segments contacting the elevated RPE, which is closely similar to the histopathology of drusen in humans[73, 75]. Many of the compounds present in human drusen have been found in drusen isolated from the cynomolgus monkey by immunohistochemistry and by proteomic analysis[63]. In addition, nonhuman primates have also been used in several toxicity studies to evaluate effects of drugs in relation with pharmacotherapy for AMD[76–78].

Preparation of an Eye Specimen for Successful Histopathology

It is comparatively difficult to prepare histological specimens of the eye because the eye is composed of several sub-organs, which have a variety of anatomical differences. In addition, proper orientation of the eye before and after enucleation is essential. Therefore, it is necessary to confirm the location of lesions, to remove the eye carefully from the orbit, to select suitable fixatives, to trim the ordered position and/or the lesion site and to select embedding material for appropriate preparation of the eye specimen. Accurate and detailed information of ophthalmoscopic and gross findings plays an important role in determining the orientation of ocular lesions[5]. A selection of fixatives is essential to minimize artificial damages and artifacts in the specimen[5, 79–82]. Several fixatives are used for the eye; however, there are advantages and disadvantages of the fixatives for each sub-organ in the eye. An eye fixative that combines glutaraldehyde and formaldehyde is recommended in general toxicity studies, because this kind of fixative is easy to prepare, enables evaluation of all sub-organs of the eye, has no problems related to waste handling and does not disturb any special histological staining[79, 80]. To expose lesions of the eye in a histological preparation, indelible marks are usually made on the detected sites in the eye. Paraffin embedding is usually used to prepare histological specimens of the eye. In addition, it has been reported that the fine architecture of the eye of the cynomolgus monkey was better preserved in glycol methacrylate (GMA) specimens than in paraffin sections[81]. Unfortunately, it is difficult to detect all ophthalmoscopic lesions in histological preparations, even if several sections are prepared from an eye. Therefore, for ocular toxicity risk assessments, comprehensive evaluations with pathological findings and results of clinical, clinicopathological and ophthalmological examinations in toxicity studies should be considered.

Conclusions

The eye is composed of a variety of tissues originating from the neuroectoderm, surface ectoderm, mesoderm and neural crest, and laboratory animals used in toxicity studies possess species-related structural characteristics. Successful histopathological evaluation of ocular lesions results from understanding the characteristics of the anatomy,

physiology and species differences of the eye in laboratory animals. In addition, accumulation of historical background data on ocular lesions in laboratory animals makes it possible to understand spontaneous lesions and compare the lesions among different strains or species, ultimately contributing to accurate evaluation and extrapolation of drug-induced ocular lesions in toxicity studies to humans. Particular technical considerations in preparation of specimens are necessary for successful histopathology of the eye. Since it is impossible to observe all ocular lesions completely in histological preparations of the eye, comprehensive evaluation with histopathology, clinical observation and ophthalmological examination is important.

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