Fundus Photography, Fundus Fluorescein Angiography, and Optical Coherence Tomography of Healthy Cynomolgus Monkey, New Zealand Rabbit, Sprague Dawley Rat, and BALB/c Mouse Retina

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Abstract

Background

A variety of experimental animal models are used in basic ophthalmological research to elucidate physiological mechanisms of vision and disease pathogenesis. The choice of animal model is based on the measurability of specific parameters or structures, the applicability of clinical measurement technologies, and the similarity to human eye function. Studies of eye pathology usually compare optical parameters between a healthy and altered state, so accurate baseline assessments are critical, but few reports have comprehensively examined the normal anatomical structures and physiological functions in these models.

Methods

Three cynomolgus monkeys, six New Zealand rabbits, ten Sprague Dawley (SD) rats, and BALB/c mice were examined by fundus photography (FP), fundus fluorescein angiography (FFA), and optical coherence tomography (OCT).

Results

Most retinal structures of cynomolgus monkey were anatomically similar to the corresponding human structures as revealed by FP, FFA, and OCT. New Zealand rabbits have large eyeballs, but they have large optic disc and myelinated retinal nerve fibers in their retina, and the growth pattern of retinal vessels were also different to the human retina. Unlike monkeys and rabbits, the retinal vessels of SD rats and BLAB/c mice were widely distributed and clear. And the OCT preformance of them were similar with human beings.

Conclusion

Monkey is a good model to study changes in retinal structure associated with fundus disease, rabbits are not suitable for studies on retinal vessel diseases and optic nerve diseases, and rats and mice are good models for retinal vascular diseases. These measures will help guide the choice of model and measurement technology and reduce the number of experimental animals required.

Background

The choice of experimental animal and measurement technology is critical for the applicability and translation of basic medical research to human diseases. Qualitative and quantitative changes in anatomical structure and physiological function are the basis of disease research, so accurate descriptions of baseline properties are essential. Numerous reports have examined normal parameters for

blood, heart, brain, liver, kidney, and other large organs among common experimental model animals [1–5], but few have examined eye structure and function in different models for ophthalmology research. A complete set of anatomic and physiological parameters for specific experimental animals will be invaluable for choosing the optimal model, preparation technique, and measurement technology. Further, high-precision measurements can increase statistical power, reduce the number of animals required, and broaden the scope of analysis to earlier or less severe conditions. Fundus fluorescein angiography (FFA) is a mainstay method for evaluating changes in retinal blood flow [6] that uses sodium fluorescein as a contrast agent to reveal vessel pattern and integrity. Continuous image capture by fundus camera allows for dynamic observation of retinal vessels during disease development for diagnosis, prognosis, and treatment evaluation. Optical coherence tomography (OCT) is a newer optical imaging technique that has shown rapid development these years [7]. It is based on the principle of weak coherent light interferometry, in which differential light scatter and reflection among individual tissue types can be used to construct two-dimensional and three-dimensional images of complex organs. It is widely applied in ophthalmology for the non-contact and non-invasive analysis of living eye microstructure, and has yielded high-resolution images of morphological structures in both the anterior and posterior segments of the eye. Therefore, OCT has important applications in the diagnosis and research of intraocular diseases, especially retinal diseases.

In this study, we performed fundus photography (FP), FFA, and OCT examinations in healthy cynomolgus monkeys, New Zealand rabbits, Sprague Dawley (SD) rats, and BALB/c mice to provide precise baseline parameters for future studies. We also suggest the best models and inappropriate models for specific applications.

Methods

1. Animals

Three conventional (CV) male cynomolgus monkeys (Guangzhou Yuanyuan Animal Breeding co. LTD, Guangdong, China) weighing ~ 4.0 – 5.0 kg, six CV male New Zealand rabbits (Huadong Xinhua Experimental Animal Farm, Guangdong, China) weighing ~ 1.5 – 2.0 kg, ten Specific Pathogen Free (SPF) male SD rats weighing ~ 180 – 220 g, and ten SPF male BALB/c mice (Jinan Pengyue Experimental Animal Breeding co. LTD, Shangdong, China) weighing 18 – 22 g were used in these studies. All animals were housed according to national standard GB 14925 – 2010 of the People's Republic of China. Monkeys and rabbits were housed under the following conditions: temperature 25 ± 1 °C, humidity 60% $\pm$ 5%, air purification Level 8, noise $\leq$ 60 dB, ammonia concentration $\leq$ 14 mg/m³, light/dark cycle 12 h/12 h. Rats and mice were housed in a barrier environment under the same temperature, humidity, air purification level, noise level, ammonia concentration, and light cycle. All animals were given free access to water and food and all were acclimated for a week before experiments. Both eyes were then examined using FP, FFA, and OCT. All animal procedures were performed in accordance with the Association for Research in Vision and Ophthalmology (ARVO) Statement for the Use of Animals in Ophthalmic and

Vision Research and approved by the Institutional Animal Ethics Committee of Zhongshan Ophthalmic Center, Sun Yat-sen University (Animal Welfare Assurance No.2016 – 120). Monkeys in this research would send back to the farm for semi-free range after experiments. And mice, rats and rabbits would proceed euthanasia after the research. They would be injected with over dose of potassium chloride solution after successful anesthesia.

2.Anesthesia

Cynomolgus monkeys and New Zealand rabbits were anesthetized by intramuscular injection of 3% sodium pentobarbital (0.5 ml/kg, Sigma-Aldrich, Darmstadt, Germany) and 0.1 ml/kg xylazine hydrochloride (Su-Mian-Xin II, Shengda Animal Drug Company, Dunhua City, China), SD rats by intraperitoneal injection of 3% sodium pentobarbital (1 ml/kg), and BALB/c mice by intraperitoneal injection of 0.3% sodium pentobarbital (0.1 ml/mg). After successful induction of general anesthesia, tropicamide eye drops (Mydrin, Alcon, Fort Worth, Texas, USA) were administrated to dilate pupils. During examination, animals were kept warm using an electric blanket and the corneas kept moist by application of artificial tears (from the pharmacy of Zhongshan Ophthalmic Center, Guangzhou, China).

3.Fundus Photography And Fundus Fluorescence Angiography

Under general anesthesia, fundus photographs were acquired centered on the macula of cynomolgus monkeys or the optic disc of New Zealand rabbits, SD rats, and BALB/c mice using a fundus camera (monkeys and rabbits: TCR-50DX, Topcon, Tokyo, Japan; rats and mice: PHOENIX Micron IV, Phoenix Research Laboratories Inc., Pleasanton, CA 94588, USA). After baseline FP, cynomolgus monkeys were injected with 1 ml of 20% fluorescein sodium (Guangzhou Baiyunshan Mingxing Pharmaceutical Company, Guangzhou, China) in 5 s via the small saphenous vein and New Zealand rabbits with 1 ml of 10% fluorescein sodium solution via the ear vein. Early-phase photographs were acquired during the first 2 min post-injection and late-phase photographs over 8 – 10 min post-injection. Rats were injected with 0.1 ml of 10% fluorescein sodium and mice with 0.1 ml of 2.5% fluorescein sodium (same supplier) into the peritoneal cavity. Early phase photographs were taken during the first 2 min post-injection and late-phase photographs 6 – 10 min post-injection.

4. Optical Coherence Tomography

We used different OCT methods but the same OCT system for all animals (SpectralisOCT, Heidelberg Engineering, Heidelberg, Germany). For retinal thickness measures, cynomolgus monkeys were scanned horizontally and vertically at the macular fovea and the average was recorded. New Zealand rabbits were scanned over a 7 × 7 mm area centered on and below the optic disc while SD rats and BALB/c mice were scanned over a 7 × 7 mm area including the center of the optic disc. For all animals, retinal thickness was

defined as the distance from the internal limiting membrane (ILM) and retinal pigment epithelium (RPE) to the Bruch membrane. For retinal nerve fiber thickness analysis, all animals were scanned in a round area centered on the optic disc.

5. Statistical Analyses

Parameters from right and left eyes were compared by paired-sample t tests while other parameters were compared by independent samples Student's t tests. Multiple means were compared by ANOVA with post hoc Bonferroni tests for pair-wise comparisons. All statistical analyses were performed using SPSS 20.0 software (IBM Corporation, Armonk, New York, USA). Data are expressed as mean $\pm$ SD. Statistical significance was set at $p < 0.05$ (two-tailed) for all tests.

Results

1. Fundus photography

The cynomolgus monkey retina (Fig. 1A) appeared similar to the human retina on fundus photographs, with a clear optic nerve head, macula, and retinal vessels. The optic nerve head was oval (vertical in the long axis) and orange – red. Its boundary was clear and the cup to disc (C/D) ratio was about 0.2. Retinal arteries were bright red, veins were dark red, and the mean ratio of artery to vein diameter (A/V) was about 2:3. The macula was located on the temporal side of the optic disc, 1.5 vertical optic disc diameters (1.5 PD) from the optic disc. It was taupe in color and free of vessels. The healthy retina was flat and even, pigmentation was symmetrical in all directions.

Figure 1B presents FPs of New Zealand rabbit retina showing the optic nerve head, retinal vessels, and choroid. The optic nerve head was oval (horizontal in the long axis) and orange – red. The boundary was unclear and the C/D ratio was about 0.4 – 0.5. Ivory-colored myelinated nerve fibers and retinal vessels were observed passing through the optic disc. Compared to retinal veins, retinal arteries were brighter and thinner (A/V of about 2:3). Healthy New Zealand rabbit retinas were flat and even, no macular-like structure and pigment were observed. The choroid was visible beneath the retina but the macula could not be distinguished.

Fundus photographs of SD rats revealed a clear optic nerve head, retinal vessels, choroid (Fig. 1C). The optic nerve head was round and light yellow. The boundary was clear but with no obvious optic cup structure. No macula was detected, too. Retinal vessels were radially distributed. Healthy retinas were flat and even, without pigmentation. Circuitous choroid vessels were observed beneath the retina.

The BALB/c mouse retina (Fig. 1D) appeared similar to the SD rat retina on fundus photographs. The optic nerve head was quasi-circular, light yellow, and exhibited no unclear boundary or optic cup structure. No macula was observed. Retinal vessels were radially distributed. Healthy retinas of BALB/c mice were flat and even, with no pigment. Choroid was observed beneath the retina but the fine structure was

unclear. New Zealand rabbits, SD rats, and BLAB/c mice are albino strains. In New Zealand rabbits and SD rats, the individual structures and distribution of choroid vessels were clearly visible beneath the retina. However, the choroid of BLAB/c mice was indistinct.

2. Fundus Fluorescence Angiography

After passing through the optic disc, the two retinal arteries of cynomolgus monkey bifurcated to form 4 arteries supplying the superior temporal, inferior temporal, superior nasal, and inferior nasal quadrants of the retina. The retinal veins ran in parallel with the arteries and converged first into upper and lower veins then a central vein before leaving the retina. The mean time from injection of fluorescein sodium into the monkey small saphenous vein to appearance in the retina was 10.7 ± 0.6 s. During the early phase of angiography (0 – 2 min post-injection), individual choroid capillaries filled with fluorescent dye and progressively became indistinguishable. Then the primary and secondary arteries filled, followed by the secondary and primary veins. The optic disc was also stained. During the late phase (8 – 10 min), fluorescence in retinal vessels faded and the optic disc appeared as a halo due to residual staining. There were no vessels at the macula, so this area remained fluorescence-free throughout image acquisition (Fig. 2A).

The two primary retinal arteries of New Zealand rabbits distributed to the nasal and temporal sides of the optic disc, emitting smaller branches along the way. Two large veins ran in parallel with the arteries, then passed through the optic disc. Circulation time from injection of fluorescein sodium into the ear vein to the retina was 8.4 ± 2.1 s. During the early phase, choroid became visible, followed by retinal arteries and veins in succession. During the late phase (6 – 10 min post-injection), vascular fluorescence faded, and optic disc fluorescence eventually disappeared. Individual choroid vessels filled with fluorescent tracer during the early phase and eventually became indistinguishable. The fluorescence faded more rapidly from choroid than from retinal vessels (Fig. 2B).

SD rat retinal vessels emerged from the optic disc, travelling straight and then radially. An average of 13.6 ± 2.3 vessels was observed in each rat retina. During angiography, retinal vessels became fluorescent rapidly and faded quickly. Due to the absence of pigment, the background fluorescence from the choroid was strong and interfered with the resolution of retinal angiography. However, vessels of the choroid may have filled and emptied rapidly compared to the acquisition rate, such that much of this process was missed (Fig. 3A).

Retinal vessels of BLAB/c mice emerged from the optic disc and formed an average of 10.7 ± 1.3 branches that distributed radially. During the early phase of FFA, retinal vessels gradually filled with fluorescein, and individual capillaries could be distinguished. During the late phase, large branches emptied but the vascular walls remained stained. Like SD rats, background fluorescence from the choroid was strong (Fig. 3B).

3. Optical Coherence Tomography

The structure of the cynomolgus monkey retina resembled that of the human retina on OCT images. These images distinguished 11 retinal layers, the retinal nerve fiber layer (RNFL), ganglion cell layer (GCL), inner plexiform layer (IPL), inner nuclear layer (INL), outer plexiform layer (OPL), outer nuclear layer (ONL), external limiting membrane (ELM), myoid zone, ellipsoid zone, photoreceptor outer segment (OS), retinal pigment epithelium and Bruch's complex (RPE/BM) [8–10], in addition to the underlying choroid (Fig. 4A). Macular foveal thickness did not differ between right eyes and left eyes ($207.17 \pm 14.77 \mu\text{m}$ vs. $209.43 \pm 5.26 \mu\text{m}$, $p > 0.05$). Alternatively, RNFL thickness differed significantly among the 4 quadrants (Table 1) ($p = 0.001$), with significant pair-wise differences between temporal and inferior ($p = 0.008$), superior and nasal ($p = 0.005$), and inferior and nasal ($p = 0.001$) quadrants.

Outside the optic disc, 11 layers were still observed in New Zealand rabbits' retina, including (RNFL, GCL, IPL, INL, OPL, ONL, incomplete ELM, photoreceptor inner segment (IS), junction of outer and inner segment, photoreceptor outer segment, and RPE/BM) (Fig. 4B) [11–13]. The average thickness of New Zealand rabbit retina was $251.20 \pm 34.78 \mu\text{m}$. Retinal thickness values within a 3 – 6 mm annular region centered on the optic disc are presented in Table 1. There was no significant difference between temporal and nasal retina thickness ($p > 0.05$). Due to the large optic disc of New Zealand rabbits, the annular region within 1–3 mm and RNFL thickness could not be measured.

Due to the multitude of retinal vessels in SD rats, many vascular shadows were observed by OCT, which partly obscured the RNFL. Nonetheless, 9 retinal layers could still be distinguished (RNFL, GCL, IPL, INL, OPL, ONL, ELM, IS/OS, and RPE/BM) (Fig. 4C) [14, 15]. Retina and RNFL thickness values across the retina are summarized in Table 1. Statistically significant differences in rat retinal thickness were found between annular regions of diameter 1 – 3 mm and 3 – 6 mm ($p = 0.000$), and between superior and inferior quadrants within the annular region of diameter 1 – 3 mm ($p = 0.01$). Mean RNFL thickness also differed significantly among the 4 quadrants ($p = 0.000$) and the inferior quadrant differed significantly from all three other quadrants (temporal $p = 0.000$, superior $p = 0.000$, nasal $p = 0.022$).

Like SD rats, numerous vascular shadows were seen on OCT images of BALB/c mouse retina. Nonetheless, 9 layers could be resolved (RNFL, GCL, IPL, INL, OPL, ONL, ELM, IS/OS, and RPE/BM) (Fig. 4D) [16]. The thickness distribution in different areas of retina and RNFL are summarized in Table 1. There was a statistically significant difference in retinal thickness between the annular region 1 – 2.22 mm from the optic disk and that 2.22 – 3.45 mm from the optic disk ($p = 0.000$), but no significant differences among quadrants within these annular regions ($p > 0.05$). Mean RNFL thickness did not differ among quadrants ($p > 0.05$).

Table 1
Thickness Distribution of Retina and RNFL (μm)

Area			Temporal	Superior	Nasal	Inferior	Mean ± SD
Monkey	RNFL		82.93 ± 4.62	124.51 ± 20.49	56.07 ± 4.62	145.56 ± 22.98	113.19 ± 16.27
Rabbit	Retina	3-6mm*	304.6 ± 65.69	163.2 ± 30.04	308.1 ± 35.18	228.9 ± 44.04	251.20 ± 34.78
Rat	Retina	1-3mm*	242.11 ± 11.55	234.22 ± 11.97	245.94 ± 11.12	258.89 ± 21.73	245.29 ± 12.19
		3-6mm*	229.72 ± 8.96	224.94 ± 8.93	226.89 ± 7.73	234.44 ± 15.27	229.00 ± 9.46
	RNFL		45.47 ± 2.45	43.56 ± 2.66	47.47 ± 2.45	51.98 ± 4.00	47.33 ± 2.58
Mouse	Retina	1-2.22mm*	215.47 ± 12.82	218.47 ± 13.46	214.44 ± 16.72	214.22 ± 11.86	215.65 ± 12.61
		2.22-3.45mm*	227.22 ± 8.71	233.33 ± 19.88	229.92 ± 17.89	234.47 ± 17.68	231.24 ± 13.74
	RNFL		39.18 ± 8.08	37.56 ± 7.06	39.76 ± 7.65	37.91 ± 8.00	38.32 ± 7.34
<p>*1-3mm: in an annular region centered on the optic disc with a diameter of 1–3 mm</p> <p>3-6mm: in an annular region centered on the optic disc with a diameter of 3–6 mm</p> <p>1-2.22mm: in an annular region centered on the optic disc with a diameter of 1-2.22 mm</p> <p>2.22-3.45mm: in an annular region centered on the optic disc with a diameter of 2.22–3.45 mm</p> <p>RNFL: retinal nerve fiber layer</p>							

Discussion

Most retinal structures of cynomolgus monkey were anatomically similar to the corresponding human structures as revealed by FP, FFA, and OCT. A notable exception was the monkey optic disc shape, which was oval with long-axis in the vertical direction whilst the human structure in more symmetrically round. The circulation time from the small saphenous vein to retina was also approximately equal to that in human. The pattern of RNFL thickness variation in different directions from the optic disc was also similar to human, with greater thickness in superior and inferior quadrants compared to temporal and nasal quadrants. Therefore, the cynomolgus monkey is a very good experimental model to study structural changes associated with fundus diseases.

The large eyeball of the New Zealand rabbit permits easy experimental manipulation, measurement, and dissection, and so is widely used in ophthalmologic research. However, FP and FFA revealed two notable

differences compared to human retina, a larger optic disc and myelinated retinal nerve fibers [17, 18]. There were also substantial differences in the growth pattern of retinal vessels compared to the human retina. Retinal vessels of New Zealand rabbits distributed in a small area of temporal and nasal optic disc, while a larger area was supplied by the choroid. The temporal and nasal areas of retina were significantly thicker than superior and inferior areas due to the myelinated fiber distribution as revealed by FP, which is also distinct from human retina. The inferior region of the rabbit retina was also thicker than the superior area. Thus, to track changes in retinal thickness among individual animals, it is critical that measurements are taken at the same location. In fact, there is substantial variation in retinal thickness among studies, which may be explained by inconsistencies in measurement location or different kinds of rabbits [11–13]. We could not analyze RNFL thickness in rabbits because the large optic disc obscured the RNFL on OCT images. In conclusion, New Zealand rabbits are not suitable for research on retinal vessel and optic nerve diseases.

Compared to monkeys and rabbits, the retinal vessels of SD rats and BLAB/c mice were widely distributed and clear. However, the smaller rat and mouse eyeballs require precise laboratory techniques and high-resolution measurements. Retinal thickness differed according to distance from the optic disc on OCT images. Therefore, to compare retinal changes at different time points within the same animal, it is critical to choose the same retinal location. In mice, RNFL thickness also differed by orientation, which may yield artifactual differences if the measurement site is inconsistent. In rats, inferior RNFL and inferior retina were thicker than the superior RNFL and superior retina, respectively, possibly due to the greater number of retinal vessels. However, due to the small eye and high vessel density, RNFL thickness is inherently difficult to measure accurately in rats and mice. Further, neural fibers and vessels were highly reflective, which interfered with OCT resolution. Therefore, we suggest that the “ganglion cell complex (GCC)” [19, 20] including the RNFL, GCL, and IPL be used as a metric for retinal ganglion cell changes in rats and mice rather than the RNFL. The GCC includes all major compartments of retinal ganglion cells, while the RNFL is mainly composed of axons, the GCL of somata, and the IPL of dendrites. Therefore, GCC is a superior metric for overall condition (but not for specific changes in individual compartments). Measurement of the GCC may also reduce errors caused by retinal blood vessel changes.

The vascular structures of the retina can also be revealed by stretched preparations, and results do not appear to differ from FFA findings [21]. However, FFA images are not influenced by operative manipulations or post-mortem changes, and can also reveal changes in retinal vessel pattern and flow over time in the same preparation. Thus, FFA is recommended for studies on retinal vessel diseases. In this research, the injected concentrations and volumes of contrast agent were obtained from literature review [22–26] and our experimental experience. At high concentrations, signals rose faster and higher to reveal the overall vascular structure quickly and in detail. However, lower concentrations offered more time for observation during the early phase.

In the three albino strains, signals from choroid were high on both FP and FFA due to the absence of pigment, which obscured the resolution and detection of retinal vessels. Therefore, albino strains are not suitable for studies on retinal vessel diseases. On the other hand, due to the lack of pigment and little

cover by retinal vessels, New Zealand rabbits may be a good model to study choroid diseases by FFA. In OCT scanning, however, no obvious differences were observed between albino and non-albino animals.

Conclusion

We examined the healthy retinas of cynomolgus monkey, New Zealand rabbit, SD rat, and BALB/c mouse by fundus photography, fundus fluorescein angiography, and optical coherence tomography to identify models most appropriate for specific experimental applications in ophthalmology. The structure of the cynomolgus monkey fundus was closest to human, and so cynomolgus monkey is the best model for examination of fundus lesions, although the cost is also highest. New Zealand rabbits have large eye balls, so experimental manipulations and measurements are easier than in other small mammal models such as mice and rats. However, fundus structure is much different from that of human, so this is an unsuitable model for fundus disease research. The retinal vessels of SD rats and BALB/c mice were clear, and retinal structure was similar to human. While the smaller eyeballs increase the difficulty of experimental manipulation and measurement, low cost and short rearing times are advantageous for experiments on retinal diseases. Interestingly, we also found the fluorescence in the retinal vessels during FFA was disturbed by the choroid in albino strains, due to the lack of pigment. Therefore, if researchers choose the rats or mice for retinal vessels diseases, they should choose the pigmented strains such as Long Evans rats or C57BL/j mice.

List Of Abbreviations

Sprague Dawley rats (SD rats), fundus photography (FP), fundus fluorescein angiography (FFA), and optical coherence tomography (OCT), conventional (CV), Specific Pathogen Free (SPF), Association for Research in Vision and Ophthalmology (ARVO), internal limiting membrane (ILM), retinal pigment epithelium (RPE), cup to disc (C/D), ratio of artery to vein diameter (A/V), vertical optic disc diameters (PD), retinal nerve fiber layer (RNFL), ganglion cell layer (GCL), inner plexiform layer (IPL), inner nuclear layer (INL), outer plexiform layer (OPL), outer nuclear layer (ONL), external limiting membrane (ELM), photoreceptor outer segment (OS), retinal pigment epithelium and Bruch's complex (RPE/BM), photoreceptor inner segment (IS), ganglion cell complex (GCC).

Declarations

Ethics approval and consent to participate:

All applicable international, national, and/or institutional guidelines for the care and use of animals were followed. All animal procedures were performed in accordance with the Association for Research in Vision and Ophthalmology (ARVO) Statement for the Use of Animals in Ophthalmic and Vision Research and approved by the Institutional Animal Ethics Committee of Zhongshan Ophthalmic Center, Sun Yat-sen University (Animal Welfare Assurance No.2016 – 120).

Consent for publication

Not applicable.

Availability of data and materials

All data generated or analysed during this study are included in this published article [and its supplementary information files].

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

BH and WHL were involved in the conception and design of the work; BKX, MLZ, WCW, ZQL were involved in the acquisition of data; BKX, YTP, HNZ were involved in the analysis and interpretation of data; BKX drafted the work; BH, WHL approved the submitted version. All authors read and approved the final manuscript.

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Figures

Figure 1

FP of cynomolgus monkey, New Zealand rabbit, Sprague Dawley rat and BALB/c mouse (A) Fundus photograph of cynomolgus monkey was centered on the macula. The optic disc (black circle) and the optic cup (white circle) was located on the nasal side of the macula (white arrow). Retinal vessels (retinal

artery – red arrow, retinal vein – yellow arrow) and retinal nerve fibers passed through the optic disc. (B) Fundus photographs of New Zealand rabbit (optic nerve head - black circle, optic cup – white circle, retinal vessels - red arrow, myelinated nerve fibers - ivory structure between the two dotted lines), SD rat (C, optic nerve head - black circle, retinal vessels - yellow arrow, choroid - green arrow) and BALB/c mouse (D, optic nerve head - black circle, retinal vessels - yellow arrow) were centered on the optic disc as no macula was detectable. Retinal vessels and retinal nerve fibers passed through the optic disc. The choroid was visible.

Figure 2

Time course of retinal vascular staining in monkey and rabbit as measured by FFA (A1) During the early phase of angiography, individual choroid capillaries filled and became indistinguishable in cynomolgus monkey. And this was followed by filling of retinal arteries. (A2-5) Retinal veins and small vessels then appeared, and the optic disc stained. (A6-9) During the late phase, fluorescence from retinal vessels faded, and the optic disc appeared as a halo. (B1-3) During the early phase, choroid vessels filled with fluorescein (B1), followed by retinal arteries (B2) and veins (B3) in New Zealand rabbit. (A4-8) Fluorescence faded quickly in the choroid (A4-6), followed by washout of veins and arteries (A7, A8). The optic disc did not stain (A9).

Figure 3

Time course of retinal vascular staining in rats and mice as revealed by FFA (A) During the early phase of angiography, retinal vessels filled rapidly (A1-5) and faded quickly (A5-9). (B) During the early phase, retinal vessels filled gradually (B1, B2), and individual capillaries became distinguishable (B3-7). During the late phase, large branches emptied but vascular walls remained stained (B8, B9).

Figure 4

OCT of retina in cynomolgus monkey, New Zealand rabbit, SD rat and BLAB/c mouse (A1) The corresponding area of OCT in the fundus photograph in cynomolgus monkey. (A2) The macular area of cynomolgus monkey retina in OCT scanning. (A3) The round area centered on the optic disc and the scanning picture of OCT. (B1) Corresponding area of OCT scanning in the fundus photograph of New Zealand rabbit. (B2) Inferior area of optic disc in New Zealand rabbit. (C1) The corresponding area of OCT in the fundus photograph of SD rat. (C2) Superior area of the optic disc and corresponding area in the fundus photograph. (C3) The round area centered on the optic disc and the scanning picture of OCT. (D1) Corresponding area of OCT scanning in the fundus photograph of BLAB/c mouse. (D2) Inferior area of the optic disc and corresponding area in the fundus photograph. (D3) The round area centered on the optic disc and the scanning picture of OCT. RNFL: retinal nerve fiber layer, GCL: ganglion cell layer, IPL: inner plexiform layer, INL: inner nuclear layer, OPL: outer plexiform layer, ONL: outer nuclear layer, ELM: external limiting membrane, IS: photoreceptor inner segment, IS/OS: junction of outer and inner segment, OS: photoreceptor outer segment, PRE/BM: retinal pigment epithelium and Bruch's complex.

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