· Review ·

Methods for making animal models of retinal vein occlusion

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视网膜静脉阻塞动物模型的制作方法

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摘要

视网膜静脉阻塞(RVO)分为视网膜分支静脉阻塞和视网膜中央静脉阻塞,是以视网膜静脉扩张迂曲、血流瘀滞、出血和水肿为特征的病变,常并发黄斑水肿和新生血管,新生血管型青光眼是其最严重的并发症。视网膜静脉阻塞对视力危害较大,是仅次于糖尿病性视网膜病变的第二大致盲性眼病。目前为止,视网膜静脉阻塞的患病人数增多,但其发病机制尚未完全明了,而且也无长久有效的治疗方法。实验室中动物模型对视网膜静脉阻塞发病机制和治疗方法的研究至关重要,因此本文对视网膜静脉阻塞实验中使用的动物及模型的制作方法做了简要综述,并对各种视网膜静脉阻塞动物模型的优缺点进行讨论。
关键词:视网膜静脉阻塞;实验动物模型;激光光凝

Abstract

• Retinal vein occlusion (RVO) is divided into branch retinal vein occlusion and central retinal vein occlusion. It is characterized by retinal vein dilatation and tortuosity, blood flow stasis, bleeding and edema. It is often accompanied by macular edema (ME) and neovascularization. Neovascular glaucoma is the most serious complications. RVO is the second most common cause of visual loss classified under retinal vascular disorders after diabetic retinopathy. So far, the number of patients suffering from retinal vein occlusion has increased, but the pathogenesis of retinal vein occlusion has not been fully understood and there are no treatments that are very long-lasting. The research of animal models on the pathogenesis and treatment of the RVO is very important. Therefore, this article gives a briefly review to the animals and model making methods used in retinal vein occlusion experiments, and discusses the advantages and disadvantages of various RVO animal models.
• KEYWORDS: retinal vein occlusion; experimental animal models; laser photocoagulation
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INTRODUCTION

Retinal vein occlusion (RVO) is a disease characterized by retinal varicose veins, stagnation, bleeding and edema. It is often accompanied by macular edema (ME) and neovascularization. Neovascular glaucoma is the most serious complications[1]. ME is usually an important cause of vision loss. Long-term ME often causes irreversible damage to vision[2]. The causes of retinal vein occlusion are complex, it often caused by a variety of factors, such as changes in blood vessel walls, changes in blood fluidity, and changes in hemodynamics. At present, anti-VEGF therapy is the first-line therapy of RVO-ME[3], but the curative effect cannot be maintained for a long time[4], finding more and better treatments is necessary for RVO.

Animal models provide researchers with the opportunity to study disease mechanisms at any stage of the pathological process, and they can also be used for cellular and subcellular research techniques that are not suitable for clinical research. RVO animal model is an induced disease animal model,

which can overcome the interference of slow human disease, long incubation period, and various etiology. It can also use a single etiology to replicate a typical animal disease model in a short time. The establishment of an animal model of retinal vein occlusion can facilitate researchers to observe the entire process of retinal vein thrombosis and its complications, and it can also facilitate the observation of the clinical efficacy of various drugs. Therefore, animal models are important for the study of the pathogenesis and optimal treatment of retinal vein occlusion. The establishment of appropriate experimental animal models provides better opportunities for the study of retinal vein occlusion. A suitable RVO animal model should consider the selection of animal breeds and strains, the simplicity and repeatability of model making, and similarities to human function and metabolic morphology. In experiments, rodents and mammals are often used as models of retinal vein occlusion, this article gives a briefly review to the animals and model making methods used in retinal vein occlusion experiments, and discusses the advantages and disadvantages of various RVO animal models. All types of experimental animals have been approved by the local animal ethics committee, and are in line with ARVO's statement on eye-use animals and vision studies.

RVO Model of Murine Murine are small rodents and are often used in experiments. Ebneter *et al*[5] established RVO model using BALB/c AnNCrl mice, and used OCT technology to study the morphological changes of the retina in RVO model; Fuma *et al*[6] established a retinal edema model using ddY mice, and studied the effects of anti-VEGF antibodies on edema and retinal unperfusion; Hida *et al*[7] and Nishinaka *et al*[8] established a ddY mouse model to study the effects of ROCK inhibitor and kallidinogenase on retinal edema and non-perfusion area, with a view to finding new methods for multiple treatments; Uddin *et al*[9] established a C57BL/6 RVO mouse model and applied the HYPOX-4 probe to study the relationship between retinal hypoxia and the occurrence and development of ischemic vascular disease. In these experiments, the early intervention and anesthesia methods were similar when the RVO model was established, the difference is the drug concentration of dilated pupils, the injection Rose Bengal, and the power and duration of the laser. Male mice of 5-8wk old are used in the experiments. Murine were placed in a controlled temperature and humidity environment at 23℃±3℃, and provided food and water. After a 12h light/dark cycle (from 08:00-20:00), the murine are anesthetized by intramuscular or intraperitoneal injection of a mixture of ketamine (120 mg/kg) and xylazine (6 mg/kg), or Inhale anesthesia with isoflurane. When Ebneter *et al*[5] established the RVO model of BALB/c AnNCrl mice, the pupils were dilated with 0.5% tropicamide/2.5% phenylephrine eye drops, RVO was induced by 532 nm laser with a power of 160 MW (duration: 0.8-2.5s; spot diameter: 50 μm) photocoagulation using a slit lamp adapter mounted on a commercially available slit lamp within 3min after intravenous injection of 0.15 mL rose bengal (4, 5, 6, 7-tetrachloro-2', 4', 5', 7' -tetraiodo-fluorescein, 5 mg/mL saline) into the tail vein. In most eyes, multiple burns are required to reach the blockage of retinal blood vessel. Burns are performed from the distal end to the proximal end in order to reduce the risk of iatrogenic vitreous hemorrhage. An average of 2-5 burns per vein. Observation of the fundus when whitening of blood vessels and stasis at the distal end are considered as complete occlusion. When the RVO model was established with ddY, C57Bl/6J, BALB/c mice and C57BL/6, tail vein injection of Rose bengal (8 mg/mL), C57BL/6 mice were injected with 40 mg/kg Bengal rose, and use an image-guided laser system (532 nm) attached to a Micron IV retinal imaging microscope (Phoenix) with a power of 50 MW, duration: 5000 milliseconds; C57BL/6 mice: 1s; spot diameter: 50 μm. With a diameter of three optic discs from the head of the optic nerve, 10-15 laser spots were applied to the branch veins of the mouse eye for photocoagulation, which blocked the retinal veins and caused ischemia to form RVO. In experiments, it was found in the modeling process that even the same retinal occlusion technique was used, BALB/c and C57BL/6J mice are difficult to establish cystic edema, while ddY mice can establish cystic edema model. Therefore, if it is necessary to establish a mouse model of cystoid edema of retinal vein occlusion, ddY mice can be selected preferentially.

RVO Model of Rat Rats are larger rodents and are often used in experiments. Ieki *et al*[10], after establishing a model of retinal vein occlusion using 10-week-old Long-Evans male rats and a new type of photosensitizer, quantitative evaluation of the injured blood - retinal barrier was conducted to investigate the factors related to RVO injury recovery. Yuan *et al*[11] established RVO model with adult male Sprague-Dawley rats to study the effect of Fufang Xueshuantong capsule on retinal vein occlusion model; Zhang *et al*[12] established an RVO model with adult female Sprague - Dawley rats, and observed the temporal changes in retinal physiology and histology of RVO rat models to study molecules related to retinal ischemia and cell death. In the experiment, they anesthetized and dilated the pupils in a similar way. 1.5% pentobarbital sodium (0.2 mL/100 g) was anesthetized by intraperitoneal injection or ketamine (5 mL, 100 mg/mL), xylazine (2.5 mL, 20 mg/mL), acepromazine (1 mL, 10 mg/mL) composed of a mixed anesthetic by intraperitoneal injection (0.8 mL/kg) to anesthetize rats. The pupils were dilated with 0.5% procaine local anesthesia, 0.5% topocamide and 2.5% phenylephrine hydrochloride. In the experiments of Ieki *et al*[10], the new photosensitizer P-AD-S31 (10 mg/kg) was injected intravenously through a tail vein catheter 3min before laser irradiation. All rats received intraperitoneal injection of 0.2 mL 10% sodium fluorescein for FAG 5min before laser treatment. Treatment was performed using a 78-diopter non-contact lens. Continuous irradiation with a semiconductor laser (wavelength: 675 nm) occludes the retinal vein at a site one disc diameter from the edge of the optic disc in the eye. The effect of FAG on thrombosis was

observed with argon laser (wavelength 490 nm). The time to complete occlusion of each vein was measured. The coagulation output is 3 MW, and the spot size is 300mm. In the experiments of Yuan *et al*[11] and Zhang *et al*[12], they built the Sprague-Dawley rats RVO model in a similar way, the only difference was the laser exposure time. Rose Bengal, 20 mg/mL in sterile saline, was injected into the tail vein (20 mg/kg), and within 3min, the fundus of the rats was observed with a ophthalmoscope, and the retinal vein was irradiated with diode laser (532 nm) at a distance of 1.5-2.0 optic disc diameter from the optic nerve diameter. The laser parameter was set to 75 μm, and the power was 0.1w, the exposure time was used by Yuan *et al*[11] was 0.2-0.3s, the exposure time used by Zhang *et al*[12] is 0.4s. Rats usually have about 6 retinal veins. In the experiments, 3 retinal veins are often blocked by laser to form an RVO model.

Rabbit Model of RVO Rabbit eyes are anatomically similar to human eyes in size, structure, and eye examination[13], and are often used in experiments. Abdallah *et al*[14], establishing a rabbit RVO model to study ultrasound-assisted custom liposome destruction for thrombolysis to treat retinal vein occlusion, it has been confirmed that ultrasound-assisted thrombolysis is a new method for the treatment of retinal vein occlusion; Jiang *et al*[15] established a rabbit model to study the inhibitory effect of grub extract on retinal microglia after RVO; Jaime *et al*[16] established a rabbit model to study changes in blood oxygen saturation in blood vessels during acute retinal vein occlusion. In the experiment, normal rabbits weighing 2-3 kg were selected, and rabbits were anesthetized by intramuscular injection of a mixture of ketamine hydrochloride (25 mg/kg) and xylazine hydrochloride (6 mg/kg). The pupils were dilated with a topical application of phenylephrine hydrochloride 2.5% and tropicamide 0.5% eye drops. All rabbits underwent color fundus photography and FFA baseline imaging before the laser. Guided by baseline FFA, the 532 nm laser was applied to retinal veins located at or near the disc margin. 10s after intravenous injection of Rose Bengal (40 mg/Kg), the laser was used to irradiate the nose or temporal retina main vein or both, taking care to avoid damage to adjacent arteries. The laser parameters are as follows: spot size, 125 μm; duration, 500 ms; power starts at 150 MW. After obstruction is observed, the power is increased to 300 MW to prevent immediate reopening of the vein. 10-30 laser points (mean 20 points) were applied to each vein of the rabbit until the treated vein became white at the proximal end and congested at the distal end. The formation of RVO was confirmed by FFA. In the experiment, the blocked venous vessels of rabbits will recanalize to varying degrees, therefore, if a long-term stable model of retinal vein occlusion is needed, it is necessary to consider whether to use rabbits, or use anticoagulant drugs after establishing a rabbit retinal obstruction model.

Pig Model of RVO In recent years, the international animal protection organization's restrictions on experimental animals have become more stringent. The use of non-human primates and canines has been restricted. Pigs have been used as food animals to replace some experimental animals. de Smet *et al*[17], established a farm pig RVO model to study how to use ocriplasmin to remove retinal vein occlusion. In the experiment, farm pigs aged 5-7wk were anesthetized with intramuscular injection of xylazine (2.2 mg/kg) and telimazole azepam (4.4 mg/kg). The animals were then intubated with a cuffed endotracheal tube (internal diameter of 6-7.5 mm) followed by mechanical ventilation. Maintain anesthesia with 1.5% - 2% isoflurane. 2 - 7min after intravenous injection of Rose Bengal (10 mg/kg), insert the endoscope with a 25G cannula, a 532 nm diode laser was used, the power was set to 140 MW and 110 ms, and the laser diameter was about 3-4 times the diameter of the vein. After the last branch in front of the optic nerve, the retinal vein was blocked until the venous retrograde filling confirmed the obstruction and RVO formed. In the past 10 years, mini pigs have been successfully used in animal disease models, corneal preservation and xenograft, mini pigs were also used for RVO research. Mendrinos *et al*[18] established the mini pig RVO model to investigate the effect of intravenous injection of L-lactate on the diameter of retinal arterioles in mini pig after acute BRVO, in the experiment, microinjection of L-lactate reversed arteriolar contraction that occurred during acute experimental BRVO. After intramuscular injection of 3 mL midazolam maleate (15 mg), 3 mL azaperone (120 mg), and 1 mL atropine (0.5 mg), anesthesia was induced with 2-3 mg of ketamine hydrochloride injected into an ear vein. Analgesia was induced with 2 mL (100 g) fentanyl and 2 mL (4 mg) pancuronium bromide. The animals were intubated and artificially ventilated. After the arterial, venous, and bladder catheters were inserted, anesthesia, analgesia, and muscle relaxation were maintained by continuous infusion of ketamine, fentanyl, and pancuronium, respectively, throughout the experiment. After the arterial, venous, and bladder catheters were inserted, anesthesia, analgesia, and muscle relaxation were maintained by continuous infusion of ketamine, fentanyl, and pancuronium, throughout the experiment. Ventilate the animals with a continuous flow of 20% O_2 and 80% N_2O at a rate of approximately 18 times/min with a respirator, keep their body temperature between 36 and 37 degrees Celsius with a warm blanket. The upper and lower eyelids and the rectangular area of the skin around the eye were excised, and the bulbar conjunctiva was separated. Carefully clean the sclera to 5 mm from the corneal limbus, the superficial scleral vessels were thermocauterized, and fix the eyeball with a metal ring sutured around the limbus. A dural incision was performed 2-3 mm behind the limbus. Place a small contact lens with a flat outer surface on the cornea. The pupils were dilated with 1% atropine eye drops and the fundus was observed with a surgical microscope. Passing an argon laser, its argon green wavelength is (514 nm). Vein occlusion has a power of 250 MW, a pulse duration of 0.5s, a spot diameter of about 500 μm, and the first branch of a main vein protruding from the optic nerve

head is photocoagulated until the vein is congestion.

Dog Model of RVO Tameesh *et al*[19] established an RVO dog model to observe the therapeutic effect of retinal vein intubation with Tissue Plasminogen Activator on retinal vein occlusion. In the experiment, dogs were anesthetized with 1.5 mg of Telazole and 2% of inhaled isoflurane. The pupils were dilated with 10% phenylephrine hydrochloride and 1% tropicamide eye drops. Immediately after intravenous injection of Rose Bengal (40 mg/kg), 15-20 laser points were applied to the retinal vein by diode green laser at a power of 100 to 150 MW for a duration of 0.2s. Immediately after photocoagulation, congestion of the distal vein was found, and there was a large amount of scattered retinal hemorrhage in the blood vessel running area, and RVO was formed. Nowadays, dogs are used in experiments, but they are not used much. This is because the animal protection movement and the fact that people often take dogs as companions in their lives, and they are more concerned about dogs, so the use of dogs is limited to some extent.

Cat Model of RVO Wada *et al*[20] established the RVO cat model to observe longitudinal changes in retinal blood flow in cat retinal vein occlusion models. They selected adult European shorthair cats of 2.3-3.6 kg, anesthetized with 5 mg/kg of ketamine and 0.2 mg/kg of medetomidine, and dilated the pupils with 0.5% tropicamide. A 0 diopter contact lens is placed on the cornea and a drop of sodium hyaluronate is added to protect the cornea. A contact lens is placed on the cornea, and an argon laser is irradiated onto the superior branch of the retinal temporal vein. Block the vein at a disc diameter of 1.0-3.0 from the edge of the disc to avoid damaging adjacent arteries. The laser spot size is 200 μm, the duration is 0.2s, and the power is 300-500 MW. 20-30 shots of the selected vessel are performed until a blocked vein is observed. Hayashi *et al*[21] established a RVO cat model to study the effects of retinal vein occlusion on protein tyrosine phosphorylation, angiogenic growth factor production and signal protein activation in the retinal tyrosine kinase pathway, it was found that protein tyrosine phosphorylation may play an important role in vascular endothelial cell mitosis and other retinal responses after RVO. de Juan *et al*[22] studied the effect of ischemia on mitotic activity of capillary endothelial cells during retinal vein occlusion, they selected adult male cats weighing 2-3 kg, anesthetized with ketamine (40 mg/kg) and acepromazine (1.6 mg/kg), and then pupils were dilated with 1% tropicamide and 10% phenylephrine. Under the operating microscope, select one eye to make a small incision in the conjunctiva, and use a 20 gauge needle to penetrate the vitreous cavity through the incision. With the help of a contact lens on the cornea, a bipolar coaxial diathermy probe is inserted into the vitreous, adjust the current to the lowest level that causes retinal vein contraction, one or two major retinal veins were coagulated about at 1 disc diameter apart from the optic disc, take care to avoid adjacent retinal arteries. After retinal vein occlusion, the proximal vein turns white from the point of occlusion, and the distal vein appears tortuously dilated. The probe was then removed from the eye and an 8-0 silk suture was placed to close the incision. Gentamicin (5 mg) was injected into the subconjunctival cavity, and antibiotic ointments were applied to the cornea and conjunctiva. Cat eyes were periodically observed after surgery with indirect ophthalmoscope. From the 1940s-1970s, the use of cats in experiments expanded. Later, the animal models represented by mice gradually matured, and the proportion of cats used in experiments gradually decreased. In recent years, the number of experimental cats has been on the rise, but cats are often treated as pets in life, and experimental cats are often under pressure from public opinion. Moreover, it is difficult to raise cats in a centralized manner, and the cost is high. Therefore, fewer cats are used in experiments.

Monkey Model of RVO In 1965, there is still a lot of gaps in the knowledge of central retinal vein occlusion, Hayreh *et al*[23] established the RVO model of rhesus monkeys to study the characteristics of central retinal vein occlusion. In rhesus monkeys, orbitotomy was performed to expose the central retinal vessels, the vessel were then blocked by diathermy near the site where they entered the optic nerve sheath from the orbit, the RVO model was established. Virdi *et al*[24] and Hayreh *et al*[25] established the RVO model of cynomolgus monkeys to study the pathogenesis of neovascularization in the eye. Cynomolgus monkeys have four major branches of the retinal vein. They photocoagulated three of the veins as close to the optic disc as possible by argon laser, taking care not to damage the accompanying retinal arteries. Rhesus and cynomolgus monkeys, as non-human primates, have long life cycles and biological characteristics that are very similar to humans. They are the best experimental materials for studying human chronic diseases. They can observe the occurrence and development of diseases and complications, and can be effective assess the efficacy and safety of the drug. In terms of retinal vein occlusion, rhesus and cynomolgus monkeys can be used to observe the pathogenesis of retinal vein occlusion and its complications, and can also find more treatments for retinal vein occlusion.

Caprine Model of RVO Caprine have also been used to simulate retinal vein occlusion, but there are few applications, and found only one case in the literature. Chen *et al*[26] used an intraocular electrocoagulation - induced caprine RVO model in the experiment, to study the feasibility of retinal vascular bypass surgery. The power of the electrocoagulation device was set to a continuous mode of 8-10, and electrocoagulation was blocked at the first branch of the blood vessel in the main retina vein. To confirm that the treated blood vessel was completely occluded, a methylene blue solution was injected into the retinal blood vessel through the ophthalmic artery. After the blockage, the distal branch veins were blue and the main veins were light red. The RVO model was established.

DISCUSSION

The incidence of retinal vein occlusion is increasing year by

year[27]. Currently, monoclonal antibodies and fusion proteins of anti-VEGF drugs are used in the clinic, it has made great contributions to the treatment of macular edema due to retinal vein occlusion, but some patients still have recurrent macular edema and non-perfusion zone after injection, and anti-VEGF drugs often require repeated injections, which adds financial burden to patients[28]. Choroidal retinal vein anastomosis and other operations are also used clinically, but the treatment effect is still controversial[3]. At this time, animal models are particularly important. There are many pathogenesis of retinal vein occlusion. Animal models can target more pathogenesis and seek more and better treatment methods, which will help to obtain new methods and models of RVO treatment.

In the experiments, murine and rats are inexpensive, easy to obtain, easy to feed and manage, and the retinal arteries and veins are clear, suitable for making large samples of RVO models for pathophysiology research. However, the eyeballs of rats and murine are too small to be suitable for surgical treatment, and neither rat nor murine has a macula, and a macular edema model cannot be established. Rabbits are cheap, readily available, easy to breed and manage, and their eyes are anatomically similar to human eyes in size, structure, and eye examination, with stable surgical and FFA conditions. However, the retinal blood supply system of rabbits is quite different from that of humans. Moreover, the macular of rabbits is inconsistent with humans, and it is difficult to establish a macular edema model. The neuroanatomical and vascular aspects of pig eyes are close to the human retina, the dog was chosen as an animal model because its retinal vessel diameter and the way the blood vessels exit from the optic disc are similar to humans, but neither pigs nor dogs have macular; The morphological distribution of cat retinal blood vessels has many similarities with humans, and the cat's eyeballs are larger, which facilitates morphological observation and surgical operation. Unfortunately, cats also do not have macular; Caprine are also used to make RVO models, but they are used less often in experiments. The retinal blood supply system of primate macaques is very close to that of humans. The retinal blood vessel morphology and distribution are very similar to the human eye. In addition, the capillaries of monkeys are more dense in macular area, they have a well-developed capillaries arch ring structure in macular area, and the center is a foveal avascular zone. Not only can a simple retinal vein occlusion model be created, but also a retinal vein occlusion macular edema model. However, monkeys are expensive and difficult to obtain, and are not suitable for large sample RVO experiments.

In clinical practice, many patients are not treated until complications such as macular edema occur. However, except for non-human primate, the remaining animals did not have macula. Pigs are similar to humans in terms of anatomy, physiology, and disease mechanism, and the mini pigs used in the experiments are small in size, small in consumption of feed, short in generation, and have the dual characteristics of large domestic animals and experimental animals. In addition, pig eyeballs have retinal blood vessels, nerve distribution and scleral thickness similar to human eyes. They are relatively suitable animals for RVO experiments at present, and mini pigs are expected to be used in large quantities. Although animal models are of great help to the research of RVO, animals are different from humans. Most animals do not have a complete central retinal vascular system and no macular. And animal models are mostly young and healthy, when the models were made, retinal vein occlusions suddenly occurred on the basis of healthy blood vessels. Therefore, the differences between animal eyes and human eyes, as well as the differences between disease models and human disease development mechanisms should be taken into account in the experiments to properly evaluate the experimental results.

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