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OXYGEN-INDUCED RETINAL NEOVASCULARIZATION MODEL IN NEONATAL RATS AND ITS VALIDATION

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Background: Severe cases of retinopathy of prematurity (ROP) are mainly manifested by retinal neovascularization, retinal hemorrhage, vitreous hemorrhage, etc., which seriously affects the visual functions of the patients. It is necessary to develop animal models of related diseases when exploring the pathogenesis of human ROP and screening and validating the efficacy of new drugs for ROP and diabetic retinopathy. Retinal neovascularization (RNV) is one of its characteristic pathological changes. Oxygen-induced retinopathy (OIR) model is one of the commonly-used animal models used in ROP pharmocodynamic research. Currently in preclinical CROs in China, there is a lack of OIR models used in pharmacodynamic evaluation. JOINN Suzhou has established neonatal rat OIR model to be used in pharmacodynamic research of new drugs.

Purpose: Inhibit the growth of retinal blood vessels in young SD rats in a high-oxygen environment, and then transfer the young rats to a normal environment (relatively low-oxygen environment) to induce retinal proliferative diseases and generate new blood vessels. Establish the separation and spreading techniques of the isolated retina of young rats, and use the adenosine diphosphatase activity staining method to evaluate retinal neovascularization. Verify the feasibility of the neonatal rats OIR model with the aflibercept intraocular injection (Eylea®) available on the market. Provide new drug R&D services for pharmaceutical companies and research institutions in China and abroad through the development of OIR model in neonatal rats.

1. Establishing Animal OIR Model

Method: Purchase SD rats in three batches.

The first batch: 4 litters of pregnant rats. 3 litters were put in the oxygen box, with 17 rats per litter. The remaining litter had fewer than 17 rats, put in normal environment serving as replacement.

The second batch: 3 litters of pregnant rats. 2 litters were put in the oxygen box, with 17 rats per litter. The remaining litter had fewer than 17 rats, put in normal environment serving as replacement.

The third batch: 3 litters of pregnant rats. 2 litters were put in the oxygen box, with 17 rats per litter. The remaining litter had fewer than 17 rats, put in normal environment serving as replacement.

Modeling condition for preliminary use: Mark and weigh all the neonatal rats born on the day (D1), eliminate small or oversized rats and mix all the rats that are moderately weighted. Divide the rats into 17 per cage and put the caged animals into the oxygen chamber of the Attendor animal gas control system. Set the system oxygen control to 7 cycles of 50% for 24 hours and 10% for 24 hours (Figure 1).

Figure 1 Attendor animal gas control system and oxygen box, with aninals in the box

On D15, take the animals out and put in normal environment. On D21, euthanize the rats and collect eyeballs, immerse the eyeballs in 10% neutral formalin (NBF) for 5-10 minutes; dissect and separate the retina and keep the retina in 10% NBF overnight. Then stain the retina with adenosine diphosphatase activity staining method.

Staining method is as follows: 1) Wash the retina for 3 times in room temperature with 50mM Trizma Maleate buffer solution, 10 minutes each time; 2) Incubate the retina under 37oC with ADPase stain for 15-20 minutes; 3) Wash the retina for 3 times in room temperature with 50mM Trizma Maleate buffer solution, 10 minutes each time; 4) Soak the retina in ammonium sulfide for 1-2 minutes; 5) Wash the retina once with 50mM Trizma Maleate buffer solution, and keep the retina in the buffer solution under 4oC; 6) Lay the retina on a glass slide coated with mounting glue for mounting, and place the retina slide in a dark place to dry. Then use the digital slice scanner (NanoZoomer-S600) to scan and observe RNV (see Figure 3).

Figure 2 OIR Staining result of Adenosine Diphosphatase activity in the retina of neonatal rats

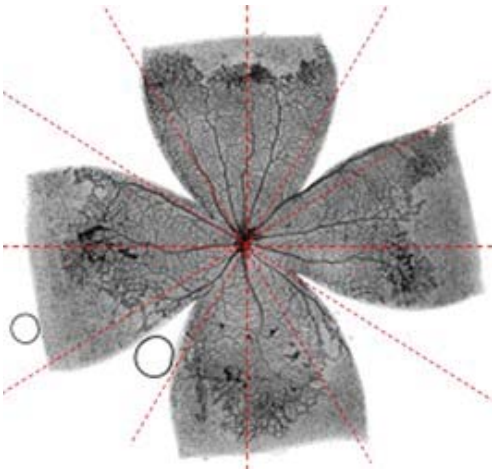

Figure 3 Illustration of 12-hour Division OIR Neonatal Rat Retina Spread

Result: The modeling conditions were determined (the oxygen level in the oxygen box is set to: 7 cycles of 50% for 24 hours and 10% for 24 hours), and the key details of the dyeing method are optimized (the eyeball is soaked in 10% NBF for a fixed time which is adjusted to 2.5h-3h, and the time that the retina is immersed in ammonium sulfide is 5-15 seconds). Once the conditions were mature, the third batch of modeling experiments was carried out, and the results showed that more than 85% of the eyes could develop retinal neovascularization.

2. Model Validation

Method: Purchase 5 litters of newborn rats in two batches (2 litters in the first batch, 3 litters in the second batch). Select 17 newborn rats (1 litter, 1 female rat)of moderate weight (5-7g) from the first batch and put into the animal gas control system for oxygen induction modeling. Oxygen level is set to 7 cycles of 50% for 24 hours and 10% for 24 hours. This is set as D1. On D15, randomly divide the 13 surviving rats into 2 groups, the model control group and the Eylea® as group, with 6 or 7 animals in each group respectively. The Eylea® group was injected with Eylea® into the vitreous cavity of both eyes (20mg/mL, 5 μL/eye) and the control group was not injected. The second batch of 34 newborn rats (17 per litter, 2 female rats) were modeled in the same way. On D15, the surviving 34 rats were divided randomly into 3 gropus, model control group, Eylea® Group A and Eylea® Group B, with 12,12, and 10 animals in each group, respectively. The control group was not administered Eylea®. Eylea® Groups A and B were administered Eylea® in both eyes (20mg/mL, 5 μL/eye on D16 and D19. On D22, both batches were euthanized. Eyeballs were taken out, retina was separated, spread, and scanned. Use the 12-hour position method to score RNV (see Figure 3).

Result: The RNV scores of the control group and the model group in the first batch were 3.1±2.6 and 1.0±1.8, respectively, with no statistical significance. In the second batch, the RNV scores of the control group, model group with D16 drug administration and model group with D19 drug administration were 4.1±3.2, 1.6±1.4, and 1.8±1.7, respectively. Scores of the model groups were significantly lower than the control group (Table 1).

Table 1 Scores of Adenosine Diphosphatase Activity Staining of Neovascularization in Neonatal Rats

Group	Dose (mg/eye)		Score
Control	0	$\bar{X} \pm SD$	4.1±3.2
		n	24
Eylea®-D16	0.1	$\bar{X} \pm SD$	1.6±1.4 *
		n	24
Eylea®-D19	0.1	$\bar{X} \pm SD$	1.8±1.7
		n	20

Table 1 Scores of Adenosine Diphosphatase Activity Staining of Neovascularization in Neonatal Rats ($\overline{X} \pm SD$)

Group	Model Group N=24	Eylea® 0.1μg/eye	
		D16 N=24	**D19** N=20
Score	**4.1±3.2**	1.6±1.4*	**1.8±1.7***

Note: *refers to compaing with model control group, p≤0.05.

Conclusion: Under the conditions of this study, intravitreal injection of eylea® (0.1mg/eye) has a significant inhibitory effect on oxygen-induced retinal neovascularization, which is consistent with the pharmacodynamics or clinical effects of Eylea®, indicating that this OIR rat model can be used for the pharmacodynamic research on the treatment of ROP in premature infants.