Animal Models Used to Simulate Retinal Artery Occlusion: A Comprehensive Review

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Purpose: To present an overview of animal models of retinal artery occlusion (RAO).

Methods: Through a systematic literature search in PubMed and Embase, papers describing methods of inducing RAO in animal models were included. The identified methodologic approaches were presented in a narrative synthesis and compared with RAO in humans.

Results: In total, 83 papers reporting on 88 experiments were included. Six different species were used with rodents and monkeys being the most common, and a minority were performed using cats, dogs, rabbits, or pigs. The anatomy of pigs and monkeys resemble that of humans most closely. The two most frequently used methods were laser-induced occlusion or ligation of the arteries. Other methods included raised intraocular pressure, arterial clamping, administration of vasoconstricting agents, the use of an occluder, embolization, and endovascular approaches to induce occlusion. In general, occlusions lasted for only 30 to 90 minutes, often followed by reperfusion.

Conclusions: Although a broad range of methods have previously been used, they all have limitations. Preferably, the methods should imitate the human disease as closely as possible and avoid damaging other structures. Therefore, monkeys followed by pigs are to be preferred and ligation or clamping may be a suitable model in larger animals as there is a potential to isolate and occlude the retinal artery only. Being less invasive, laser-induced occlusion is another suitable approach.

Translational Relevance: This review aims at assisting researchers in deciding on the most ideal experimental setting, and thereby increase the translational value to human disease.

Introduction

Retinal artery occlusion (RAO) can cause severe and irreversible vision loss. RAO is divided into branch retinal artery occlusion (BRAO) and central retinal artery occlusion (CRAO) based on the site of arterial occlusion. It is most often caused by an embolism resulting in infarction of the inner retina and may be regarded as the ocular analogue to cerebral stroke.1,2 Various treatment strategies for the acute management have been explored, such as ocular massage and thrombolysis; however, no treatment has yet been shown to be effective.3 In order to develop treatment that can limit the extent and severity of ischemic injury, a deeper understanding of the pathophysiology and biochemical processes is needed. Experimental animal models of RAO have the potential to broaden our knowledge of the disease in general and lead to the development of new
treatments in particular. Also, retinal tissue is accessible in animal models allowing for advanced analyses directly at the retinal level where RAO is located.

Numerous experimental animal studies of RAO have previously been performed using a variety of different methodologic approaches with varying similarities to human conditions. However, the use of different models for inducing illness and disparate animal species, among other factors, makes it difficult to compare and extrapolate findings with humans. Indeed, criticism has been raised against several of the previously employed methods. Improvement and standardizations of the scientific method followed by systematic review may increase the translational value to human disease.

Therefore, the aim of the present paper was to present a comprehensive overview of previously applied methodologic approaches that can be used for conducting animal models of RAO in order to assist future researchers in deciding on the most ideal experimental setting.

**Methods**

For this review, a systematic literature search was conducted in PubMed and Embase (Supplementary Tables S1 and S2). Prior to the search, a protocol was written. Studies were included if their experimental method induced occlusion of the retinal artery, regardless if their primary aim was to set up a model for RAO or not. The retrieved studies were examined to exclude overlapping or duplicated data. Experimental models of occlusion of vessels more proximal than the ophthalmic artery were excluded, as these more closely resembled manifestations of other diseases, such as ocular ischemic syndrome.

For each included study, the species used in the study was noted, method of inducing the occlusion of the retinal artery, regardless if their primary aim was to set up a model for RAO or not. The retrieved studies were examined to exclude overlapping or duplicated data. Experimental models of occlusion of vessels more proximal than the ophthalmic artery were excluded, as these more closely resembled manifestations of other diseases, such as ocular ischemic syndrome.

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**Species**

A number of different species have been used (Table 1). Rodents are the most commonly used species in the experimental animal models, the second most frequently used species being monkeys. A smaller number of experimental setups included cats, dogs, rabbits, or pigs. Especially, dogs, cats, and pigs were used in studies using methods requiring larger animals (occluder/probe, endovascular techniques, or embolization).

The resemblance to human anatomy varies widely among the species. In monkeys, the vascular architecture is very similar to humans. Furthermore, monkeys have a macula as in humans, as opposed to the other species included. Overall, the structure of the retinal vascular system of the pig is similar to that of humans too. Diverging results exist regarding the presence of a central retinal artery (CRA) in the pig. Some authors report of a single retinal artery giving rise to several branches, while others report of no formation of a CRA. The findings suggest that the retinal arteries may either arise from the ciliary artery as several branches, or as a single branch, which quickly divides. Once the nerve head is reached, only branches are visualized, typically four.

In contrast to humans and the other species used, both cats and dogs have a tapetum lucidum, which is a reflective layer improving vision in dim light. In the cat, three major arteries and two to six smaller arteries run from the optic disc to the periphery. In the dog, there is no CRA. Instead, multiple vessels pierce the sclera, branching off usually three or four retinal arteries. As opposed to humans, the arteries show tortuosity.

The retinal vascular system of the rabbit eye is distinctly different from the human eye and the other species used in the identified studies as the rabbit retina is merangiotic (presence of blood vessels in a limited part of the retina leaving the rest of the retina avascular). Furthermore, the retinal circulation of the rabbit may play little role in maintaining retinal function as monitored by the electroretinogram (ERG), as suggested by Ciulla et al.
Method of Inducing RAO

Overall, RAO was induced by several methods, including laser, vasoconstriction by injection of chemical substances, increased intraocular pressure (IOP), embolization, or arterial occlusion by using a clamp or suture mainly, or by using an occluder (Table 1 and Fig. 2).

### Laser

Sixteen studies used laser photocoagulation (Table 2). It was most often performed using an argon laser with wavelengths varying between 514 to 577 nm. Often, a photosensitizing agent was used to increase laser absorption and minimize the amount of laser required to produce occlusion. Hence, nine studies

### Table 1. Animal Species and Techniques Used to Produce Occlusion

| Technique            | Rodents, n | Rabbits, n | Cat, n | Dog, n | Pig, n | Monkey, n | Combination, n | Total, n | References       |
|----------------------|------------|------------|--------|--------|--------|------------|----------------|----------|-----------------|
| Laser, n             | 14         | 4          | 0      | 0      | 0      | 4          | 1              | 23       | 7–18,28–37      |
| Vasoconstriction, n  | 2          | 3          | 0      | 0      | 0      | 0          | 1              | 0        | 6,16,38–42      |
| Raised IOP, n        | 9          | 1          | 0      | 0      | 0      | 0          | 1              | 0        | 11,43–53        |
| Clamp, n             | 0          | 0          | 0      | 0      | 0      | 9          | 0              | 9        | 5,18,54–60      |
| Ligation, n          | 20         | 1          | 0      | 0      | 0      | 3          | 0              | 24       | 38,61–83        |
| Occluder, probe, n   | 0          | 0          | 5      | 0      | 0      | 0          | 1              | 6        | 24–27,84        |
| Embolization, n      | 0          | 1          | 0      | 2      | 3      | 0          | 0              | 6        | 19–23,85        |
| Endovascular technique, n | 0       | 0          | 0      | 0      | 3      | 0          | 0              | 3        | 86–88           |
| Total, n             | 45         | 10         | 5      | 2      | 6      | 18         | 2              | 88       |                 |

Figure 1. Flow chart of study selection procedure.
used rose bengal and seven studies used no photosensitizer.

Six studies used photodynamic therapy or a photodynamic therapy–like method. In these studies, a photosensitizing agent was administered intravenously or, in one study, intraperitoneally followed by application of laser or another light source for a duration varying from 2 to 45 minutes. One study used laser to induce targeted delivery of a platelet-activating agent, in this case adenosine diphosphate.

If a CRAO was induced by laser, the laser beam was oriented at the optic disc. If one or more BRAO was aimed for, then the laser beam was targeted at one or more arterioles, typically near the optic disc.

Vasoconstriction

Apart from one study using serotonin, vasoconstriction was induced by administration of endothelin-1, which is a potent vasoconstrictor produced by vascular endothelial cells. Generally, endothelin-1 was either injected in the posterior vitreous body over the optic disc, which causes constriction of all retinal vessels, including both veins and arteries, or it was injected in the subconjunctival space in the posterior part of the eyeball causing constriction of the CRA. It is not evident if this last method causes constriction of other vessels too.

Serotonin was used in one study to test the hypothesis that serotonin may cause vasospasm in atherosclerotic monkeys. In total, nine monkey eyes were used, and in four of these an occlusion of the CRA was evident, an additional two showed slight filling delay.

Raised IOP

Most frequently, occlusion or ischemia was produced by cannulating the anterior chamber with a tube connected to an elevated reservoir containing saline. The IOP is then elevated to typically 120 mm Hg (range, 90–160 mm Hg). In one study, the IOP was increased by inserting and inflating an arterial embolectomy catheter balloon retrobulbarly to simulate orbital hemorrhage. This resulted in occlusion of the CRA in two of 16 monkeys.

Clamp

This method consisted of performing a lateral orbitotomy followed by clamping of the CRA at its site of entry into the optic nerve dural sheath. It was performed on monkeys in all nine studies using this method.

Ligation

For this procedure, one study used a clip, the rest used a ligature. For the large majority, the procedure of ligating the CRA in rodents included ligation of the optic nerve and other vessels (posterior ciliary artery). However, two studies using rodents reported applying ligature solely on the CRA, only one of them with a detailed description on how it was done. The optic nerve was exposed by blunt dissection after a lateral conjunctival dissection and removal of the lateral rectus muscle. The CRA was exposed by longitudinally opening the dural sheath surrounding the optic nerve using fine scissors. The suture on the CRA was positioned after the trifurcation of the ophthalmic artery into the CRA and two posterior ciliary arteries.

All three studies on monkeys placed a ligature on the CRA only.

Occluder/Probe

In studies using an occluder or a probe, the probe or occluder was either produced from a steel needle or from a glass probe tipped with a glass ball. For heat coagulation, the probe was made by sealing tungsten wire into a glass capillary tube with epoxy.

Two studies used a probe and in an identical fashion. A puncture was made through the superior lateral portion of the pars plana through which the blocker probe was inserted. BRAO was then produced by pressing the probe on a branch artery. By
pressing the probe on the optic nerve, it occluded the entire retinal circulation.\textsuperscript{25,84}

Three studies used an occluder and followed the same procedure. That is, to produce a BRAO the ball was pressed onto an artery emerging from the optic disc.\textsuperscript{24,26,27}

In one study, including two cats, the probe was used to perform heat coagulation.\textsuperscript{24} The success of occlusion was evaluated by inspecting the occluded vessel by direct ophthalmoscopy.

**Embolization**

Studies that applied embolization-based methods were carried out by injecting various substances in either the carotid artery\textsuperscript{22,23,85} or the maxillary artery.\textsuperscript{19–21}

In three related studies, BRAO was produced in 6 of 27, 12 of 26, and 8 of 33 cases by injecting platelet aggregates, fibrin clots, or leucocyte aggregates, respectively.\textsuperscript{19–21} These experiments also produced microinfarctions and hemorrhages.

Ciulla et al.\textsuperscript{22} used human atherosclerotic material, the injection of which produced BRAO in five rabbits and CRAO in four rabbits out of a total 12 animals. In one study, the injection of air produced BRAO in all animals if they were kept hypotensive.\textsuperscript{23} However, the occlusions lasted less than 10 minutes.

Long-acting corticosteroids were injected in one

| Table 2. Parameters of Laser Photocoagulation for Each Study |
|-------------------------------------------------------------|
| **Type of Occlusion** | **Photosensitizer** | **Laser** | **Wavelength, nm** | **Power, mW** | **Spot Size, \( \mu m \)** | **Duration, s** | **Number of Applications, \( n \)** | **Reference** |
|----------------------|---------------------|-----------|---------------------|--------------|-----------------|----------------|-------------------|----------------|
| BRAO                 | -                   | NA        | NA                 | NA           | NA              | NA              | NA                | 18             |
| CRAO (and CRVO)      | -                   | Argon     | NA                 | 900          | 500             | 0.2–1           | 20                | 33             |
| CRAO (and CRVO)      | -                   | Argon     | NA                 | 900          | 500             | 0.2–1           | 20                | 34             |
| BRAO                 | -                   | Blue–green argon laser | NA | 100–200 | 50 | 0.1–0.2 | Median 44 | 10 |
| CRAO                 | Rose bengal, 20 mg/kg | Argon dye laser | 577 | 80 | 50 | 0.1 | 10–20 | 13 |
| BRAO                 | -                   | Argon dye laser | NA | 150–250 | 100 | 0.5 | 3–10 | 14 |
| CRAO                 | Rose bengal, 20 mg/kg | Argon green laser | 532 | 100 | 75 | 0.4 | NA | 30 |
| CRAO                 | Rose bengal, 0.1 mL | Diode laser | 532 | 490 | 300 | 0.300 | NA | 35 |
| CRAO (all arteries) | Rose bengal, 50mg/kg | NA | 532 | 650 | 75 | 0.5 | NA | 37 |
| BRAO                 | Rose bengal, 100 \( \mu L \) 1% | Diode laser | 532 | NA | 75 | NA | NA | 15 |
| CRAO                 | Rose bengal, 20 mg/kg | NA | 514 | 100 or 150 | 200 | NA | NA | 31 |
| CRAO                 | Rose bengal, 0.05 mL of 2.5 mM YAG laser | 514 | 100 or 150 | 200 | 0.1 | 20 | 29 |
| CRAO                 | Rose bengal, 20 mg/kg | NA | 532 | 150 | 200 | NA | NA | 36 |
| CRAO                 | Rose bengal, 20 mg/kg | NA | 532 | 300 | 200 | 0.3 | NA | 16 |
| BRAO                 | -                   | Red laser light | NA | 50 | 50 | 3.0 | 2-3 | 17 |

CRVO, central retinal vein occlusion; NA, not available/not applicable.
Combined with epinephrine, this consistently produced RAO and choriocapillaris occlusion.

**Endovascular Technique**

Three studies using endovascular techniques were identified. All three studies were conducted by Morén et al. and used pigs as experimental animals. The arteries were catheterized using a transfemoral, endovascular approach.

In one study, transient and permanent vascular occlusions were performed using an angioplasty balloon catheter in the ophthalmic artery or a liquid embolic agent that was administered via an injection catheter. The liquid embolic agent could produce occlusion of either the ophthalmic artery or the main ciliary artery from which the retinal arteries branches. Occlusion of the main ciliary artery produced complete ischemia, in contrast to the ophthalmic artery, which only produced incomplete ischemia.

In the other two studies, vascular occlusion was produced by using coils in the ophthalmic artery.

**Evaluation of Successful Occlusion**

In total, 41 of 88 included studies validated their occlusion by the use of fundus examination, while 29 studies used ERG as a measure of the function of the retina. Thirty-three studies used angiography (either fluorescein angiography or indocyanine green angiography), and 33 studies used histopathology to evaluate differences in the architecture of the retina. Twelve studies did not use any of these methods.

A few studies used other methods to validate or evaluate the occlusion, such as visual-evoked potential or observing whitening of the iris and the loss of the red reflex. Optical coherence tomography was not performed in any of the studies.

**Duration of the Occlusion**

When using a setup with ligation, clamp, or increased IOP, the duration of the occlusion was controllable. The applied durations are listed in Table 3. Typically, the occlusion lasted 30 to 90 minutes followed by a varied period of reperfusion.

Endovascular approach was permanent, except when using balloon catheter, in which case the artery was occluded for 1 hour.

When inducing an occlusion by laser, the duration of the occlusion has a methodologic limit as spontaneous reperfusion occurs at a point, either due to recanalization or development of collateral circulation. Ten of 23 studies using laser reported the duration of the occlusions (see Table 4). Large differences existed between studies with the duration varying from 3 hours to 2 weeks before reperfusion. In many cases, there were no data on the exact time of reperfusion, but intervals, minimums, or maximums were given for the duration of the occlusion. One study induced reperfusion by infusion of recombinant tissue plasminogen activator.

Studies using an occluder or probe maintained the occlusion for periods ranging from 10 minutes to 4 hours.

Constriction caused by endothelin-1 infusion was in one study reported to last for approximately 5 minutes after which the effect declines, other studies found it to last for at least 50 minutes. Injection in the subconjunctival space caused constriction for 30 minutes.

One study using embolization by injecting leucocyte aggregates and subsequently exposing the animals to hypoxia reported the occlusions to last up to 8 days. Studies using other agents reported occlusions lasting up to 166 seconds or 20 minutes.

**Discussion**

Experimental animal models offer a unique opportunity to investigate different aspects of RAO. However, caution is needed when findings are extrapolated to the disease in humans. In order to increase the usefulness and applicability of RAO experiments, it is of utmost importance to imitate the human disease as closely as possible. Ideally, the anatomy of the animal should resemble that of the human eye, the intervention should mimic the occlusion of the retinal artery meaning the occlusion of the retinal artery only, and avoid damage on other tissues or structures.

Imitating the disease requires thorough knowledge on the natural history of RAO. In this aspect, the duration and extent of the occlusion is of interest. In a fluorescein angiography study in which patients were seen 4.4 days in average after the onset of symptoms, only one in 62 patients with CRAO showed complete absence of dye in the retinal arteries. However, the arteriovenous transit time was found to be prolonged in most cases. Hence, while there seems to be consensus that in the clinical setting some residual circulation remains, the mechanism is still debated. It has been suggested to be due to an incomplete obstruction by the embolus in the vessel. A study by Hayreh and Jonas on clamping of the CRA in monkeys reported of fluorescein angiographies similar to that in humans showing residual circulation.
after CRAO. It was argued that it was due to anastomoses with the CRA distal to the occlusion allowing filling, that being cilioretinal capillary anastomoses and pial and intraneural anastomoses.\textsuperscript{54}

This mechanism is only possible if the site of occlusion is proximal to the site of these anastomoses (i.e., the dural sheath). Experimental methods using increased IOP, ligation of multiple vessels, and vasoconstriction causes complete obstruction of the vessel(s) leaving no residual circulation, in contrast to the clinical picture in man.

Due to time delay, the clinical findings at the very onset of the occlusion is largely unknown. Although it is uncertain when it begins, reperfusion ultimately occurs in the majority of clinical cases. Hence, one study found reperfusion to appear in all cases of

Table 3. Duration of Occlusions and Reperfusion for Studies Using Ligation, Clamping, or High IOP

| Duration, Occlusion | Reperfusion Duration | Reference |
|---------------------|----------------------|-----------|
| NA                  | None                 | 79        |
| 30, 60, 120 min, 4, 24 hr | None               | 80        |
| 30 min              | 180 min              | 83        |
| 30–90 min           | 30 min–24 hr         | 82        |
| 90 min              | 4 or 24 hr           | 76        |
| 30 min              | 180 min              | 66        |
| 30 min              | 120 min              | 67        |
| 60 min              | Up to 3 d            | 72        |
| 30 min              | NA                   | 68        |
| 30 min              | NA                   | 69        |
| 1, 5, 10, 20, 30, 60, 120 min | NA                | 81        |
| 90 min              | 1, 3, 5 min, 24 hr   | 77        |
| 30 min              | 240 min              | 70        |
| 30 min              | 240 min              | 71        |
| 60 min              | 6, 24, 72 hr         | 64        |
| 60 min              | 7 d                  | 73        |
| NA                  | NA                   | 38        |
| 90 min              | 120 min              | 61        |
| NA                  | NA                   | 65        |
| 60 min              | 5 d                  | 74        |
| 90 min              | 24 hr                | 78        |
| 30 or 90 min        | 3 or 12 hr           | 62        |
| 60 min              | 10 d                 | 75        |
| 30 min              | 14 d                 | 63        |

| Duration, Occlusion | Reperfusion Duration | Reference |
|---------------------|----------------------|-----------|
| 180 or 240 min      | 2, 4, or 6 wk        | 43        |
| 45 min              | 3–48 hr              | 88        |
| Up to 60 min        | 7 d                  | 87        |
| 45 min              | 7 d                  | 63        |
| 45 m                | 6 hr–7 d             | 29        |
| 5 or 10 min         | 24 hr                | 85        |
| 50 min              | 0, 60 min, 6 or 24 hr| 44        |
| 60 min              | 1, 3, 5, 7, 14 d     | 61        |
| 90 min              | 1 d                  | 62        |
| 60 min              | NA                   | 45        |
| 60 min              | 0, 60 min, 6, 24 hr, 7 d | 84    |

Table 3. Extended

| Duration, Occlusion | Reperfusion Duration | Reference |
|---------------------|----------------------|-----------|
| 180 or 240 min      | 2, 4, or 6 wk        | 43        |
| 45 min              | 3–48 hr              | 88        |
| Up to 60 min        | 7 d                  | 87        |
| 45 min              | 7 d                  | 63        |
| 45 m                | 6 hr–7 d             | 29        |
| 5 or 10 min         | 24 hr                | 85        |
| 50 min              | 0, 60 min, 6 or 24 hr| 44        |
| 60 min              | 1, 3, 5, 7, 14 d     | 61        |
| 90 min              | 1 d                  | 62        |
| 60 min              | NA                   | 45        |
| 60 min              | 0, 60 min, 6, 24 hr, 7 d | 84    |
RAO, although in only 16 of the 29 patients with visible emboli perfusion recovered within the first month. A study on CRAO found only 15% of eyes to have transient CRAO (lasting several minutes to many hours), while 71% had permanent CRAO, and 14% had CRAO with cilioretinal artery sparing. In the vast majority of the experimental studies, the occlusion lasted only minutes to hours followed by either spontaneous or induced/intended reperfusion. Only the endovascular method produced permanent occlusion. Therefore, it could be argued there are dissimilarities between the majority of the experimental setups and the longer lasting CRAO in humans.

It has previously been argued in a study using laser-induced thrombus that animal models that depend on well-controlled reperfusion may produce pathogenic information that is less relevant to the clinical situation. It could be argued there are dissimilarities between the majority of the experimental setups and the longer lasting CRAO in humans. A disadvantage of several of the models is that they affect vessels other than the retinal artery. Endothelin-1 causes constriction of all retinal vessels, including veins. One study investigated the choroidal blood flow following the administration of endothelin-1 in rabbits. They found the choroidal blood flow to increase, maybe due to regulatory mechanisms. As for the method of increased IOP, even a moderate increase in IOP causes a reduction in blood flow in the choroid. In the majority of studies using ligature, the ciliary arteries were ligated as well. Injection of material to cause embolization was done in either the carotid or maxillary artery, which may very well have caused ischemia in tissues other than the retina. When using an occluder or probe only the inner retina is made ischemic. The same applies for clamp in monkeys, laser, and, to some extent, endovascular approach.

Some methods could produce features unrelated to RAO, too. Elevation of IOP may result in both vascular occlusion–induced ischemia and mechanical injury to the retina. Öz et al. reported that their model of increased IOP, “...has no similarity or analogy to isolated vascular occlusion such as clinical central retinal artery occlusion or ophthalmic artery occlusion.” Ligation often included ligation of the optic nerve. This induces mechanical and ischemic damage to the optic nerve and may also induce occlusion of the ciliary arteries resulting in choroidal ischemia. This may confound the cell degeneration attributed to ischemic damage from occlusion of the

### Table 4. Reported Duration of Artery Occlusion Induced by Laser Before Spontaneous Reperfusion

| Species       | Method                          | Site of Occlusion          | Reported Duration of Artery Occlusion | Reference |
|---------------|--------------------------------|----------------------------|--------------------------------------|-----------|
| Rodents       | Light + rose bengal             | BRAO                       | >60 min                              | 8         |
| Rodents       | Laser + rose bengal             | CRAO                       | ~6 hr                                | 31        |
| Rodents       | Laser + rose bengal             | CRAO                       | 3 hr to >24 hr                       | 30        |
| Rodents       | Laser + rose bengal             | RAO (all arteries)         | 8 hr–1 d                             | 37        |
| Rodents       | Laser + rose bengal             | CRAO                       | At least 24–48 hr                    | 32        |
| Rodents       | Light + rose bengal             | BRAO (and CRVO)            | <3 d                                 | 7         |
| Rabbits       | White light + rose bengal       | BRAO                       | Up to 3 d                            | 9         |
| Rabbits       | Laser + phthalocyanine          | Vein and artery branch     | 2–4 d                                | 11        |
| Rabbits       | Laser + chloro-aluminum sulfonated phthalocyanine | Medullary ray (artery, vein, and choroidal vessels) | 72 hr –1 wk | 28 |
| Rabbits and rodents | Laser targeted delivery + encapsulated photosensitizing agent | BRAO | At least 2 wk | 12 |

*For studies reporting information on the duration only.*
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