Neurosurgical Modeling of Retinal Ischemia–Reperfusion Injury

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Background: A reliable model of ischemia–reperfusion is required to evaluate the efficacy and safety of neuroprotective therapies for stroke. We present a novel reproducible pterygopalatine–ophthalmic artery ligation model of ischemia–reperfusion injury in the retina. *Methods:* Rats were subjected to ophthalmic artery/meningeal sheath ligation (OAML—standard method) or clamping of the pterygopalatine–ophthalmic artery (OAC—new method) for 30 minutes. Retinal ganglion cell (RGC) survival was assessed by prelabeling with FluoroGold (FG) (Santa Cruz Biotechnology, CA, USA) and RNA-binding protein with multiple splicing (RBPMS) at 14 days after ischemia, and all results were compared with a sham group (n = 7 in each group). *Results:* RGC density in the normal-uninjured (FG-labeled) group was 2111 ± 38 cells/mm^2 (mean ± standard error of mean) and that in the RBPMS-labeled group was 2142 ± 35 cells/mm^2. The *OAML* procedure significantly reduced RGC density to 738 ± 23 cells/mm^2 and 780 ± 41 cells/mm^2 ($P < .001$) in the FG-labeled and RBPMS-labeled groups, respectively. Similarly, OAC reduced RGC survival to 782 ± 19 cells/mm^2 and 813 ± 22 cells/mm^2 ($P < .001$) in the FG-labeled and RBPMS-labeled groups, respectively. RGC survival was similar following OAC and OAML models, suggesting that both induce comparable levels of damage. However, RGC survival in the OAC model was found to have less dispersion than OAML-induced ischemia. *Conclusions:* These results suggest that the OAC procedure is a reliable reproduction of ischemia–reperfusion injury that mimics the effects of ophthalmic artery occlusion in humans and provides a useful research model for testing manipulations directed against pathways involved in RGC ischemic degeneration. **Key Words:** Retina—ischemia—reperfusion—retinal ganglion cell—ophthalmic artery.

Introduction

Ischemia is an important process contributing to the pathophysiology of many brain and retinal diseases.[1] Retinal ischemia, due to relatively ineffective treatments, remains a common cause of visual impairment and blindness linked to various brain circulatory disorders.[2,3] In patients with complete internal carotid artery occlusion, chronic progressive ocular ischemia[4,5] leads to rubeosis iridis and retinal ischemia. When internal carotid artery occlusion occurs in patients with incomplete blood circulation in the circle of Willis, the blood flow in the ophthalmic artery (OA) reverses to supply the ipsilateral brain. This so-called steal phenomenon results in ocular ischemia.[6-9]

Under normal conditions, blood flow in the communicating arteries of circle of Willis is negligible. However, if a subject has an atypical circle of Willis, for example, missing one of the main arteries or communicating arteries or under pathological conditions such as complete

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or partial occlusion of one of the cerebral or carotid vessels, the flow can be redirected to perfuse deprived areas.[10,11] The ring-like structure of the circle of Willis is often incomplete or not fully developed. It has been found that in more than 50% of healthy brains[12,13] and in more than 80% of dysfunctional brains,[14] the circle of Willis contains at least 1 artery that is absent or underdeveloped. The most common topological variations include missing communicating vessels, fused vessels, string-like vessels, and presence of extra vessels.[15]

The retina develops as an extension of the diencephalon, and as such, blood vessels in both tissues share similar anatomical and physiological properties. Among these, the retinal vasculature possesses a blood–retinal barrier analogous to the blood–brain barrier.[16] As a result of these similarities, the study of retinal ischemia can also yield insights into the processes involved in cerebral ischemic damage.

A number of animal models and analytical techniques have been developed to study ischemic degeneration in the brain and retina. An immediate limiting factor in the choice of animal model is the variation of retinal vascular patterns across species.[2] The rat provides an advantageous model in this regard because the organization of its cerebral and retinal circulation closely resembles that of humans.[2,17] Several models of retinal ischemia currently exist in the rat; the most common consists of the selective ligature of the ophthalmic vessels (SLOVs)[18,19] and surgical ligation of the meningeal sheath, including the OA.[20] However, these methods cause inconsistent levels of injury, possibly owing to the downstream effects they have in addition to ischemic damage.[321] Furthermore, these models are very invasive, requiring dissection of the orbit area, and they can produce variable histological injury, which is difficult to quantify.[22,23] Secondary damage as a result of neurotoxicity may also contribute to RGC degeneration and will therefore confound the levels of retinal ganglion cell (RGC) degeneration attributed to ischemic damage.[24]

As yet, there is no ideal method for the study of ischemia or reperfusion injury in the retina.[20] The present study aims to improve on current models by proposing a novel extraocular technique of OAC that produces a more accurate picture of pure retinal ischemic injury damage by minimizing secondary damage. For comparison, results of this procedure are examined alongside those obtained with the ophthalmic artery/meningeal sheath ligation (OAML) model; the current standard for retinal ischemia studies.

Materials and Methods

This study used 50 female Sprague-Dawley rats (Charles River, Ontario, Canada), weighing 250-300 g, which were kept in a pathogen-free environment and cared for according to the Canadian Council on Animal Care.

Surgical Technique

Experiments were carried out using aseptic technique in conjunction with the animal use protocols of the University Health Networks, Toronto, Canada. Instruments and materials (solutions, test substances, tracers, needles, etc.) that came into contact with living tissue were sterilized to prevent infection and adverse impacts on animal welfare and study results.

Anesthesia

During all surgical procedures, animals were anesthetized using a veterinary isoflurane vaporizer system: medical-grade oxygen was used at a rate of .8 L/min to vaporize the isoflurane gas. Animals were placed in an anesthesia box and exposed to a 4% mixture of isoflurane until adjudged to be completely sedate (breathing became slow and steady, and the animal was unresponsive to a toe-pinch pain test). Animals were then moved to a stereotaxic frame, and the gas flow was diverted to the frame's attached gas mask. The isoflurane concentration was reduced to 2% during the surgical procedure, and sedation level was continually monitored by observation of breathing rate and depth, as well as by toe-pinch. Isoflurane concentration was adjusted during the procedure based on individual animal needs. Animal temperature was evaluated by rectal thermometer and was maintained at 37°C throughout the procedure with a thermal pad. After completion of the surgical procedure, isoflurane levels were reduced to 0% and animals were kept in the stereotaxic frame to breathe oxygen for several minutes before removal. Animals were then placed under a heat lamp until consciousness and mobility were fully regained before being placed in individual housing containers.

Retrograde FluoroGold Labeling

As previously described, 1 week before transient ischemic injury, animals received injections of 2% FluoroGold (Santa Cruz Biotechnology; CA; USA) (FG) into the superior colliculus, the brain target of RGCs, to prelabel all RGCs in the retina for future quantification.[20,25] Animals were anesthetized with isoflurane gas in a stereotaxic apparatus to which a Foredom Micromotor drill (Bethel, USA) with an adjustable arm was attached. Holes were drilled bilaterally into the skull overlying the superior colliculus. Injections were performed using a 10-μL Hamilton syringe actuated by a computer-controlled picopump (World Precision Instruments, Sarasota, USA). Two injections, each consisting of 2 μL of FG solution, were delivered at different depths within the superior colliculus at an injection rate of 500 nL/min. The needle was left in place for 10 minutes after each injection and slowly withdrawn to prevent reflux of the injected solutions. The cornea was moistened throughout the surgical procedure by