Chorioretinal biopsy in dogs

Ian J. Constable, Douglas H. Slatter, and Robert Horne

A simplified practical technique for biopsy of retina and choroid has been developed in dogs. A 270° circular scleral flap of 3 mm diameter is raised. The risks of chorioretinal bleeding and vitreous loss are greatly reduced by intravenous mannitol and controlled hyperventilation, with hyperoxygenation and transient, systemic hypotension under general anesthesia. Reasonable ultrastructural detail was preserved in chorioretinal specimens of 1 mm diameter. Repeat biopsies in the same eye are feasible without significant complications.

Key words: chorioretinal biopsy, retinal dystrophies, choroiditis, ultrastructure, hypotensive anesthesia

The fact that the retina and choroid in the living eye are inaccessible to tissue analysis by such techniques as electron microscopy, immunofluorescent antibody studies, and biochemistry has prevented advancement of the understanding of many diseases of these structures. Preliminary reports have been published of biopsy techniques using a full-thickness eye wall method1,2 and a transvitreal approach.3 Peyman et al.4 reported a scleral approach to one human eye. They used trephined scleral incisions with a circle of diathermy between to achieve penetration of the eye wall. A 4 mm disc of sclera, choroid, and retina was then removed. Vitreous loss occurred in every case. This technique has been considered a major surgical procedure. With the major risk of long-term complications after vitreous loss, it has therefore not found acceptance as a practical procedure for biopsy of diseased human eyes with useful vision.

We have developed a new method of transcleral chorioretinal biopsy which is greatly simplified and usually results in no vitreous loss.

Materials and methods

Twenty eyes of 14 adult dogs were used. The animals were premedicated for general anesthesia with acepromazine (0.5 mg/kg). Pupils were dilated with 1% atropine drops. Anesthesia was induced with sodium pentothal by slow intravenous injection, and the animals were intubated and initially maintained with standard nitrous oxide/oxygen/halothane mixture under spontaneous respiration. A lateral orbitotomy was performed by cutting diathermy to incise skin, orbital ligament, and temporalis muscle. Lateral conjunctiva and Tenon’s capsule were reflected to expose the sclera from the limbus almost to the optic nerve. A biopsy site was selected posterior to the equator and the exits of the vortex veins. A 270° full-thickness circular scleral incision was then made about 3 to 4 mm in diameter with a 57 Beaver blade. The resulting scleral flap was raised from the underlying choroid by blunt dissection. Two sutures of 9-0 nylon were placed through both edges of the incision prior to the biopsy (Fig. 1).

During these preliminary steps, mannitol 20% (2 gm/kg) was given intravenously over 20 min. A transfemoral aortic catheter was inserted and con-
Fig. 1. Diagram of dorso-lateral full thickness scleral incision with 9-0 nylon sutures preplaced prior to chorioretinal biopsy. rb, Retractor bulbi; lr, lateral rectus; dr, dorsal rectus.

Fig. 2. Chorioretinal defect approximately 1.5 mm diameter 4 months after biopsy. Note that site involved retinal vessels without causing significant damage.

Fig. 3, Late fluorescein angiographic picture of chorioretinal biopsy site and surrounding retina (same eye as Fig. 2). There is leakage from fibrovascular tissue in the base of the biopsy site but none from surrounding retina.

Fig. 4, Fundus photograph taken 1 week after second chorioretinal biopsy. Dark patches around both biopsy sites are photographic shadows. Arrows designate streaks of subretinal blood which cleared in 3 weeks.

Connected via a Hewlett-Packard pressure transducer to a Sanborn strip-chart recorder. Under high magnification with a Zeiss operating microscope, a site between major choroidal vessels was selected with the aid of transillumination through the cornea. Mean aortic blood pressure was reduced over a period of 10 min from 100 to 120 mm Hg to 50 mm Hg with the use of 3% to 4% halothane and intermittent positive pressure ventilation set at 20 cycles/min. Arterial blood gases and pH were measured during the period of hypotension. This procedure in combination with the mannitol induced ocular hypotension, reversed choroidal bulging, and caused the choroidal vessels to visibly decrease in size. The surface of the choroid was grasped with blunt-tipped jeweller’s forceps and tented upwards slightly. With curved Vannas’ scissors a full-thickness cut was made in choroid and retina. Under very high magnification it was then possible to pass one blade of the scissors onto...
the vitreous face or into the superficial layers of the vitreous cortex. A 1 to 1.5 mm sample of choroid and retina was excised by two additional cuts with the scissors. The specimen was immediately placed with retinal surface uppermost on a cellulose sponge and immersed in 2.5% cacodylate-buffered glutaraldehyde. The scleral flap was closed with the three preplaced sutures, and additional sutures were added where necessary. As soon as the flap was closed, the controlled hyperventilation was stopped, halothane concentration was decreased, and the systemic blood pressure was allowed to rise again. The conjunctiva, lateral orbital ligament, and skin were closed with interrupted chromic catgut sutures. The eyes were treated with 1% atropine and chloramphenicol ointment at the end of the procedure. No postoperative systemic antibiotics were used.

In two eyes repeat biopsies were carried out 4 and 8 weeks after the first procedure with the same techniques. All eyes were followed by indirect ophthalmoscopy and fundus photographs postoperatively. Fluorescein angiography was done on two animals with a Kowa fundus camera. The dogs were sacrificed at periods up to 6 months after chorioretinal biopsy. The eyes were fixed in 2.5% glutaraldehyde for 1 day followed by 10% formalin for one day and were blocked, cut, and stained for light microscopy or transmission and scanning electron microscopy.

Biopsy specimens were embedded in araldite. Thin sections were stained with uranyl acetate and lead citrate and examined with a Siemens electron microscope.

Results

In the first six chorioretinal biopsies done, mannitol was used to decrease intraocular pressure and dehydrate the vitreous without the aid of hyperventilation, hyperoxygenation, and systemic hypotension. In all six there were significant vitreous loss (up to 0.5 ml) and external bleeding. None of these eyes developed retinal detachment or other visible complications. The resulting round, sharply demarcated defect in retina and choroid did not change in the following 6 months (Fig. 2). Fluorescein angiography after 4 months showed minor leakage in the base of the wall due to small vessels present around the full-thickness nylon scleral sutures (Fig. 3).

A further 14 eyes had chorioretinal biopsies under controlled hyperventilation with systemic hyperventilation. Two eyes were biopsied on two separate occasions 4 and 8 weeks apart. Arterial blood gases measured at the time of biopsy under these conditions showed $P_{O_2}$ 420 to 500 mm Hg, $P_{CO_2}$ 12 to 18 mm Hg, and $pH$ 7.50 to 7.55. Under these conditions bleeding was minor or nonexistent. On 10 occasions no vitreous was lost, whereas on the others a slight bead of vitreous only was lost. No postoperative complications were observed in any of these eyes. The eyes subjected to repeat biopsies also
Fig. 6. Electron microscopic view of condensed vitreous fibrils (VF) on inner side of scleral flap, opposed to thicker newly formed collagen (NC) associated with wound repair. (×22,400.)

withstood the double insult without major complications (Fig. 4).

Histological examination after 4 months showed firm healing of the scleral wound, no detachment of the sharp retinal edge, and no gross disturbance of the vitreous in the immediate vicinity (Fig. 5). Several focal areas of loss of photoreceptors and photoreceptor nuclei were seen in one eye adjacent to the biopsy site. Pigment epithelial cells beneath the areas of photoreceptor loss were enlarged. The edges of the biopsy had appeared slightly pigmented ophthalmoscopically. Under the electron microscope, minor fibroblastic proliferation occurred at the vitreous surface, with deposition of collagen fibrils of 40 to 90 nm diameter (Fig. 6). This newly formed fibrotic tissue was not seen to invade the vitreous extensively, and there was no evidence of retinal traction.

Several earlier specimens were rolled up and showed artefactual detachment of the retina. In later specimens this was avoided by placing the sample on a cellulose sponge soaked in fixative under control of the operating microscope. Under these conditions acceptable preservation of retinal detail was obtained (Fig. 7) despite the fact that in most specimens there was still some bending of the receptor outer segments. However, these mechanical defects in specimen preparation were not considered likely to limit interpretation of pathological material.

Examination of the inner retinal surface showed that in most cases part of the cortical vitreous was excised with the specimen even when no vitreous was lost (Fig. 8).

Discussion

The need for a safe, practical choriretinal biopsy technique has been recognized for many years. Peyman and co-workers have previously shown that the procedure is feasible.
Fig. 7. Electron microscopic view from biopsy specimen of receptor elements. A, Survey view. (×2,600.) B, Junction of inner and outer segments. (×21,875.) C, Junction of outer segment with retinal pigment epithelial cell. (×16,875.)

We believe that the safety is greatly increased by taking a smaller (1 to 2 mm) sample of tissue. In the procedure developed in the dog, vitreous loss can be minimized or eliminated by a combination of controlled hyperventilation, hypotensive anesthesia, and intravenous mannitol. Under these conditions prior treatment of the choroid with diathermy to prevent bleeding is unnecessary.

The small specimens obtained by this method present mechanical problems in that bending of the retinal elements is commonly present. However, we consider them to be
Fig. 8. Inner surface of chorioretinal biopsy specimen showing that plane of excision includes superficial cortical vitreous. Note hyalocyte. (×10,400.)

quite adequate for electron microscopic interpretation since good fixation is reproducibly found.

The feasibility of multiple biopsies on the same eye will prove to be a valuable research technique. First, it will allow expensive higher-order animals such as monkeys or large animals such as dogs to be used in relatively small numbers to study the development of retinal diseases. Second, the problem of biological variability in onset and development of retinal dystrophies from one
animal to another of the same species will also be partly overcome. Third, the technique will allow sequential study of slowly progressive lesions in animal models by biochemical and morphological means.

We have found that this simplified chorioretinal biopsy technique may be adapted to humans. Five volunteers have undergone the procedure immediately prior to enucleation without vitreous loss or other complications. Controlled hypotension was induced for 3 to 8 min with a slow infusion of intravenous sodium nitroprusside. This standard anesthetic technique is used in many branches of surgery. Skilled anesthesiologists believe that induced hypotensive anesthesia is safe and ethical in medically fit patients for the short period of time required for this procedure.

REFERENCES


Information for authors

Most of the provisions of the Copyright Act of 1976 became effective on January 1, 1978. Therefore, all manuscripts must be accompanied by the following written statement, signed by one author: "The undersigned author transfers all copyright ownership of the manuscript (title of article) to The Association for Research in Vision and Ophthalmology, Inc., in the event the work is published. The undersigned author warrants that the article is original, is not under consideration by another journal, and has not been previously published. I sign for and accept responsibility for releasing this material on behalf of any and all co-authors." Authors will be consulted, when possible, regarding republication of their material.