Perfusion of occluded retinal veins in the cat's eye

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SUMMARY The organisation and adherence of a clot induced experimentally in the retinal vein of a cat was studied in vitro. For this purpose a retinal vein was treated in vivo with argon laser photocoagulation in 31 eyes, and each eye was removed at periods of time varying between 3 hours and 7 days after treatment. The freshly enucleated eye was then placed in a perfusion chamber with the cornea under a contact lens, and the retinal vessels were perfused under direct microscopic visualisation. It appears that the hydrostatic pressure and flow needed to perfuse the obstructed vein has to be increased with elapsing time, and that 7 days after laser treatment the retinal vessel becomes permanently occluded.

Vein thrombosis is a common clinical disorder in the retina as well as in other vascular areas. The treatment of this disorder could be helped by the development of an animal model to study in vitro the properties of a clot induced in life. It appears that the cat's eye could be used effectively for this purpose, since it has been shown that enucleated cats' eyes can be readily perfused through the ophthalmociliary artery, and through it both the choroidal and the retinal circulations can be activated simultaneously.^{1,2} When the corneal surface is placed under a Goldmann type of contact lens and a microscope, the blood filling the retinal vessels can be seen to be washed out by the perfusate. It occurred to us therefore that a clot induced in vivo in the retinal vein of the cat by means of argon laser photocoagulation could be studied in terms of its organisation and its adherence to the vessel wall after the eye is enucleated and perfused in vitro.

The pressure and flow needed to wash out the occluded vessel in relation to the age of the 'clot' will be reported in this study.

Materials and methods

Thirty-one adult cats' eyes were used in this study. The animals were anaesthetised with ketamine hydrochloride.

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In order to occlude a retinal vein argon laser photocoagulation was delivered to a selected vessel. The laser spot size was of 200 μm , the duration 0.2 s, and the energy 300 to 500 mV. Twenty to 25 such spots were delivered on the blood column of the selected vessel until the blood flow was seen to cease in the treated area. The artery in the vicinity was not injured by the beam. Immediately following the treatment the vein tract beyond the laser marks was seen to become engorged, and the circulation was shunted away from the area drained by this vessel. Haemorrhages in the area drained by this vessel appeared in about 50% of the cases 24 hours after this treatment.

Fig. 1 Diagram of the perfusion chamber 1. Eye platform. 2. Goldmann contact lens. 3. Movable column supporting the globe with tube inserted in a groove.

Fig. 2 Before perfusion the normal retinal vessels are filled with blood.

Fig. 3 The normal vessels washed out by the perfusate.

Three, 6, 12 hours and 1, 2, 3, 5, and 7 days after laser photocoagulation selected eyes were enucleated and used to check their patency by means of perfusion of the vessels.

For this purpose the animals were again anaesthetised and a large temporal orbitotomy was done in order to visualise the optic nerve. The 4 rectus muscles were cut off near their insertion to the sclera. The posterior pole of the globe was displaced forward with the help of a special spoon, and the optic nerve was sectioned with scissors under a microscope, leaving an 11 to 13 mm stump of nerve and the ophthalmociliary vessels attached to the eye globe.^{1,2} The enucleated eye was then placed on the concave platform of a chamber (Fig. 1) with the corneal surface pressed gently against the concave surface of a Goldmann contact lens (Fig. 2) and a drop of 2% methyl cellulose between the cornea and the lens

surface to avoid air bubble penetration. The tip of a PE 20 polyethylene tube was thinned by stretching it over a small flame and then it was cut in an oblique fashion. The tube was inserted under pressure on the thin groove of a movable column (Fig. 3), with the tip of the tube protruding about 5 mm from the front of the platform. A plastic sponge was glued to the front surface of this column, so that when the platform was moved forward the sponge pressed gently on the posterior pole of the eye globe and held the eye firmly in place. The other end of the tubing was connected to a bottle of Hartmann's solution through a PE 60 polyethylene tube. The height of the bottle could be changed to vary flow and hydrostatic pressure. The optic nerve with its vasculature was manoeuvred to be in front of the tip of the tube and the cilioretinal artery was cannulated, and tied around the tube with a 7-0 silk suture to prevent fluid leakage.

Fig. 4 Relationship between hydrostatic pressure and patency of the veins. $\blacktriangle$ Normal veins. $\circ$ Clot washed out. $\bullet$ Clot remained.

Fig. 5 Area of the fundus showing vein occlusion. Note haemorrhages around the occluded vein.

Fig. 6 Perfusion washed out the nontreated vessels. The clot remains in place.

The perfusion chamber was then set up in a vertical fashion, and the corneal contact lens was placed under an operating microscope with coaxial illumination to focus on the occluded retinal vessels and over a large area of the fundus.

The eyes were perfused from a bottle containing Hartmann's fluid. The bottle was raised gradually 5, 15, 40, 50, 100, and 200 cm height above the level of the perfused eye. The flow was of 1.5 ml/min for the lower value of pressure and 5.6 ml/min for the highest (Fig. 4). At the end of each experiment a 10% fluorescein solution was injected into the ophthalmociliary artery to demonstrate the patency or the persisting obstruction of the treated retinal vein. All the experiments were monitored through an operating microscope, and fundus photographs, video tape recording, and cinematography (16 mm) were taken occasionally through the operating microscope during appropriate stages of the experiment. Selected eyes were fixed in 10% formalin for light microscopy and in a mixture of 1% formaldehyde and 1% glutaraldehyde in phosphate buffer for electron microscopic studies of the occluded vessels. These pathological studies will be reported separately.

Results

FOUR NORMAL EYES

The normal retinal vessels of the freshly enucleated cat's eye were easily washed out with the perfusion fluid at 5 cm hydrostatic pressure and a flow of 1.5 ml/min. Fig. 2 shows the retinal vessels filled with blood, and Fig. 3 the same vessels almost empty of blood in the course of a perfusion experiment. No heparinisation of the blood was needed prior to enucleation, since blood will not clot in the retinal³ or the choroidal vessels of the cat. Unusually the vortex vein draining the choroidal circulation may become

spontaneously clotted, but cutting this vessel flush with the sclera allows for the ready flow of the perfusate.

THIRTY-ONE EYES TREATED BY ARGON LASER

These eyes were perfused between 3 hours and 168 hours after laser induced venous occlusion. At 5 cm pressure the perfusate washed all the nontreated sectors of the retinal vessels in all these eyes. However, for the treated veins there was an almost linear relationship between the hydrostatic pressure of the perfusate needed to wash out the treated vein and the time elapsed between treatment and enucleation.

It is clear from Fig. 4 that in eyes perfused 7 days after laser treatment the patency of the vein could not be re-established. Even with the perfusate at 200 cm height (5.6 ml/min flow) only in 1 out of 8 eyes could the obstructed vein be reopened. Fig. 5 shows an area of the fundus with a 7-day-old vein occlusion, and Fig. 6 shows the same area after perfusion. It can be seen that the clot remains in the vessel while the rest of the nontreated vasculature has been washed out by the perfusate.

When at the end of the experiment a 15% fluorescein solution was injected from a syringe at a very high pressure, the dye did not fill the occluded veins except in the one eye mentioned above.

Discussion

Perfusion experiments on enucleated eyes are usually done after heparin is injected in vivo into the circulation. It appears from this study in which no heparin was used that blood does not clot spontaneously in the retinal and choroidal vessels, and it is possible to wash out the blood of these vessels with a perfusate. This phenomenon is consistent with the observation that the endothelial cells of retinal vessels produce

fibrinolytic factors that inhibit clot formation.³ On the other hand it was observed in these series of experiments that occasionally the vortex veins were clotted, preventing the outflow of the perfusate, and this resulted in an elevated intraocular pressure. Although choroidal vessels have been reported to lack fibrinolytic activity,³ it appears from this study that, if the vortex veins are patent or are cut off flush with the sclera, the choroidal circulation is also not impaired *in vitro*.

Argon laser photocoagulation of a retinal vein is known to produce retinal lesions that reproduce well vein thrombosis, including retinal haemorrhages, nonperfusion areas, and subsequently shunting vessels between the occluded and nonoccluded retinal veins.⁴ In our experimental set-up we induced a venous occlusion *in vivo* by means of argon laser photocoagulation and checked the patency of the treated vessel in a perfusion chamber under direct observation with appropriate magnification.

Our results indicate that fresh 'thrombi,' formed within hours after laser treatment, are brittle enough to enable us to 'wash' them out of the vessel with a flow of 1.8 ml/min, which is mildly higher than for the control, nontreated vessels. Both hydrostatic pressure and flow must be progressively increased to perfuse the treated area of the vein as time elapses after laser treatment.

Seven days after treatment the clot in the vein appears to be sufficiently organised and adherent to

the vessel wall to resist passage of the perfusate.

Dr Michaelson often expressed his opinion that a reduced arteriolar flow could have a role in initiating a retinal vein occlusion.⁵ The present set of experiments indicate the important roles of flow and perfusion pressure in altering the physical structure of a vein obstruction. Furthermore, it appears that in order to prevent a thrombus from organising in an irreversible way it would be necessary to institute therapeutic measures in the very early stages of its formation. The effectiveness of several treatment modalities is now being investigated with the help of the cat's perfused eye model.

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