Vitreous body oxygen tension following experimental branch retinal vein obstruction

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We obstructed either temporal or nasal superior and inferior retinal veins in one eye of rhesus monkeys with xenon photocoagulation. This resulted in large areas of nonperfused retina adjacent to normal retina in the same eye. Intraretinal neovascularization (new vessels in the retina) developed following the absorption of retinal hemorrhage and edema. We used microelectrodes to measure and compare vitreous body oxygen tensions over the nonperfused and over the normal retinal areas. There was no significant difference between the oxygen tension measurements in the same eye.

Key words: branch retinal vein obstruction, vitreous body oxygen tension, intraretinal neovascularization, rhesus monkeys, photocoagulation, microelectrodes, vitreous body

Experimental obstruction of one or two major temporal retinal veins or a single retinal vein plus impairment of arterial perfusion in the same quadrant with the use of xenon or argon laser photocoagulation produces intraretinal neovascularization (new vessels in the retina) in rhesus monkeys.1–2 The new retinal vessels sprout into the nonperfused retina from the perfused bordering vasculature and grow toward the center of the nonperfused retinal area. The stimulus for retinal neovascularization is unknown, but tissue hypoxia has been implicated.3–4 We photocoagulated retinal veins in rhesus monkeys, causing formation of areas of nonperfused retina and intraretinal neovascularization, and measured vitreous body oxygen tensions.

Material and methods

We tranquilized six male and female rhesus monkeys with intramuscular phencyclidine (Sernylan), 2.0 mg/kg, and anesthetized them with intravenous pentobarbital sodium. After dilating the pupils with 10% phenylephrine hydrochloride (Neo-Synephrine) and 1% cyclopentolate hydrochloride (Cyclogyl), we obstructed the retinal veins with a xenon photocoagulator (Clinitex). We burned the retina adjacent to either the temporal superior and inferior or the nasal superior and inferior retinal veins near the optic disk. After partial occlusion, we burned directly on the vessel, causing complete occlusion. We performed fluorescein angiography on the eyes 30 min to 6½ months after photocoagulation. If large venous collaterals developed between nasal and temporal veins, we occluded them with further photocoagulation.

We anesthetized the animals from 2½ to 6½
Fig. 1. Fluorescein angiogram (monkey 10718) of a large area of nonperfused retina showing early intraretinal neovascularization. The background fluorescence indicates normal perfusion of the choroidal vasculature.

Table I. The effect of branch retinal vein obstruction on retinal oxygen tension*

<table>
<thead>
<tr>
<th>Monkey</th>
<th>Duration of obstruction</th>
<th>Normal retina (mm Hg)</th>
<th>Retinal venous obstruction (mm Hg)</th>
<th>Difference† (mm Hg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>10234</td>
<td>6½ mo</td>
<td>34 ± 8</td>
<td>36 ± 7</td>
<td>-2</td>
</tr>
<tr>
<td>10520</td>
<td>2½ mo</td>
<td>16 ± 2</td>
<td>13 ± 1</td>
<td>+3</td>
</tr>
<tr>
<td>10616</td>
<td>4 mo</td>
<td>20 ± 2</td>
<td>16 ± 5</td>
<td>+4</td>
</tr>
<tr>
<td>10718</td>
<td>3½ mo</td>
<td>15 ± 2</td>
<td>15 ± 1</td>
<td>0</td>
</tr>
<tr>
<td>10417</td>
<td>5 mo</td>
<td>15 ± 2</td>
<td>13 ± 1</td>
<td>+2</td>
</tr>
<tr>
<td>10627</td>
<td>30 min</td>
<td>13 ± 2</td>
<td>14 ± 2</td>
<td>-1</td>
</tr>
</tbody>
</table>

* Each number is the mean of a minimum of three measurements of oxygen tension taken 100 μm above the internal limiting membrane with the standard deviation.
† According to the paired t test of significance between the two means (5 degrees of freedom) p > 0.3.

months after the initial photocoagulation with 100 mg/kg of a 10% solution of alpha-chloralose in polyethylene glycol administered intravenously. We further prepared the animals for our microelectrode measurements as we have previously described. In brief, we observed the ocular fundus by axial illumination with an operating microscope through a plastic chamber which replaced the cornea. We used two micromanipulators to place and control the microelectrodes in the eye.

We determined the position of the tip of the microelectrode by placing it on the internal limiting membrane of the retina as signaled by a dimpling of the membrane. We then recorded oxygen tensions above the internal limiting membrane in the vitreous body. We have previously described our polarographic method of microelectrode oxygen tension measurement. For the studies herein reported we used a glass-insulated, gold-plated, recessed tip electrode (No. 723; Transidyne General Corp., Ann Arbor, Mich.). The electrodes are metal-filled micropipettes in which the glass envelope protrudes beyond the end of the metal approximately 7 to 10 μm. Gold is plated onto the metal filling, and it is covered by an oxygen-permeable cellulose membrane to prevent poison-
Vitreous body oxygen tension after vein obstruction

Fig. 2. Photomicrograph (monkey 10234) of nonperfused retina showing thinning and loss of structure. The retina is artifactually detached from the normal appearing choroid. (×160.)

ing. The tapered tip is 2 to 3 μm in diameter. We measured the voltage-current relationship (polarogram) of each microelectrode we used, and the current usually reached a plateau between −0.75 and −0.95 volts. We selected a polarization voltage in the middle of the plateau and calibrated the electrode with nitrogen gas, 5% oxygen, and 10% oxygen in normal saline before and after insertion into the eye. The currents we measured in the eyes were on the order of 10 picoamps and our amplifier was the Transidyne General Corp. Model 1201.

We alternated our microelectrode measurements between normal retina and the center of the area of nonperfused retina. We obtained a minimum of three measurements from each area 100 μm above the internal limiting membrane in the vitreous body. By using a polygraph with a paper speed of 2.5 mm/sec and 5 sec time marks, we recorded all the parameters. We enucleated the eyes just before the animals were sacrificed and fixed them in glutaraldehyde for preparation for light microscopy.

Results

Following the absorption of retinal hemorrhages and edema, fluorescein angiograms showed intraretinal neovascularization similar to that noted clinically (Fig. 1). It is important to note that the new vessels never entered the vitreous body.

We measured vitreous body oxygen tensions from 30 min to 6½ months following branch retinal vein obstructions. According to the paired t test, there was little significance between the two means (Table I).

There was loss of structure and marked thinning of the retina after 6½ months (Fig. 2). The choroid and its vasculature, however, appeared normal in the center of the areas beneath the nonperfused retina.

Discussion

Vitreous body oxygen tension measurements close to the retina are believed to reflect retinal tissue partial pressures. This seems all the more likely over the nonperfused retinal areas since there is less tissue utilizing oxygen. Nonetheless, the measurement of oxygen tension in the vitreous is a compromise but retinal tissue measurements are confounded by tissue destruction, un-
known electrical currents, and the difficulty of knowing the precise position of the electrode.

The sensitivity of the microelectrode is critical for the interpretation of the results. We were able to read a change in PO₂ of approximately 2 mm Hg, depending on the calibration curve. This is probably a correct estimation of the sensitivity of our measurements, since there was no measurable drift over the relatively short periods of our data collection. The recessed and coated oxygen microelectrode does not respond as fast as a bare wire but it still is only a matter of seconds. The oxygen microelectrodes are not sensitive to pH but they are temperature-dependent.

Intraretinal new vessels follow occlusive vascular disease of the retina in branch retinal vein obstruction as well as diabetic retinopathy. The new vessels are of capillary size and require fluorescein angiographic techniques for visualization (Fig. 1). They may develop from any part of the retinal vascular bed and are identified as linear capillary-like outgrowths with spikelike advancing tips. The rate of growth of the new intraretinal vessels is only a few microns per month. The vessels have normal structure and do not leak fluorescein. They are in striking contrast to the poorly constructed and incompetent vessels found in preretinal neovascularization and the relationship between these two forms of vessel growth is unknown. Indeed it is significant that branch retinal vein obstruction by photocoagulation in the experimental animal does not produce preretinal neovascularization. This suggests that there may be another factor besides vascular occlusion present in the eyes of patients who develop neovascularization following branch retinal vein obstruction.

We believe that the reason the oxygen tension is normal instead of close to zero in the center of the nonperfused retinal areas is that there is loss of the entire thickness of the retina in these areas and the intact choroidal circulation furnishes the oxygen (Fig. 2). Cell death must be extensive by 30 min, since the 30 min vitreous oxygen tension measurements were approximately the same as those obtained after several months (Table I). The choroidal circulation appeared normal under the nonperfused retina in fluorescein angiograms and histologically (Figs. 1 and 2). Furthermore, we tested the competency of the choroidal circulation in an eye with a nonperfused nasal retinal area and a normal fovea centralis. We believe that oxygen tension measurements in the capillary-free fovea centralis reflect the choroidal circulation. The animal briefly inspired 100% oxygen and the increment increase in retinal PO₂ had the same latency and amplitude in the nonperfused nasal retinal area and in the fovea centralis.

We did not measure oxygen tensions along the borders of the nonperfused areas, which might be expected to be relatively ischemic. New intraretinal vessels, however, continue to grow toward the centers of the nonperfused areas and appear to be induced by a factor or factors associated with the centers rather than the borders of these areas. It is possible that following venous occlusion there is a transient tissue hypoxia triggering intraretinal neovascularization but the vessels continue to grow for many months into tissue apparently not hypoxic. We have furnished evidence which casts doubt on the hypothesis that intraretinal neovascularization is secondary to chronic hypoxia. We have no information about the presence or absence of a diffusible vasoproliferative substance.

REFERENCES


