Dorzolamide Increases Retinal Oxygen Tension after Branch Retinal Vein Occlusion

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PURPOSE. To study the effect of dorzolamide on the preretinal oxygen tension (RPO2) in retinal areas affected by experimental branch retinal vein occlusion (BRVO) in pigs.

METHODS. Experimental BRVO was induced by diathermy close to the optic disc. RPO2 was measured with an oxygen-sensitive electrode 0.5 mm above the BRVO-affected area, which was compared to the retinal areas not affected by BRVO. In one group of five pigs, RPO2 was measured at baseline, 1 and 3 hours after BRVO, and after intravenous injection of 500 mg dorzolamide. In a second group of five pigs, RPO2 was measured 1 week after the BRVO, both before and after intravenous injection of 500 mg dorzolamide.

RESULTS. The average baseline RPO2 was 2.64 ± 0.09 kPa (mean ± SD). In the BRVO-affected areas, RPO2 decreased significantly (by 0.67 ± 0.29 and 0.94 ± 0.13 kPa) at 1 hour and 3 hours after BRVO induction. In the non-BRVO areas RPO2 increased significantly (by 0.51 ± 0.14 kPa) 1 hour after BRVO induction, but subsequently decreased and reached baseline 3 hours after BRVO induction. One week after BRVO induction, RPO2 was 0.67 ± 0.29 kPa lower in affected areas when compared with the non-BRVO areas. In the BRVO-affected areas, dorzolamide increased RPO2 significantly (by 0.56 ± 0.21 kPa at 3 to 4 hours and by 0.67 ± 0.40 kPa) 1 week after BRVO induction.

CONCLUSIONS. Retinal hypoxia induced by experimental BRVO remained significant 1 week after BRVO. Dorzolamide increased retinal oxygen tension in the BRVO-affected areas both at 1 hour and 1 week after experimental BRVO in pigs. (Invest Ophthalmol Vis Sci. 2008;49:1136–1141) DOI:10.1167/ iovs.07-0508

In healthy experimental animals, systemic administration of the carbonic anhydrase inhibitor, dorzolamide, has been shown to increase oxygen tension in the retina and the optic nerve head.1–3 In the present study, we examined the effects of systemic dorzolamide on preretinal oxygen tension, (RPO2), in a porcine model of retinal ischemia. Branch retinal vein occlusion (BRVO) is well known to cause ischemia in humans, where decreased microvascular retinal blood flow has been demonstrated by scanning laser Doppler flowmetry.4 Experimental BRVO offers a unique opportunity to compare ischemic retinal areas with nonischemic areas in the same eye. An experimental BRVO model has been established in cats and minipigs using diathermy and laser photocoagulation.5–7 In these animals, experimental BRVO has been shown to decrease oxygen tension in the area of the retina drained by the occluded vein as well as in the adjacent vitreous.5–7 In the BRVO models, scatter laser photocoagulation and vitrectomy have been shown to increase RPO2.5,6,7 The effects of the systemic carbonic anhydrase inhibitor, acetazolamide, has also been investigated in the porcine model, albeit only for acute BRVO, where intravenous administration was found not to affect preretinal oxygen tension significantly in the BRVO-affected areas.6 In combination with carbogen breathing, however, systemic acetazolamide did normalize preretinal oxygen tension in the ischemic areas.6

In the immediate post-BRVO period of these studies, the effects of retinal ischemia is confounded with the effects of surgical trauma and inflammation, as well as secondary arteriolar vasoconstriction.10–14 In the present study, we tested the effect of dorzolamide 1 week after the induction of BRVO, as well as in the acute period. One week after induction of BRVO, the acute effects will probably have decreased, and the ischemia may more directly reflect the effects of BRVO. However, the effects of vitreoretinal procedures and ocular trauma on ocular blood flow may be diverse and last much longer than 1 week.15,16

MATERIALS AND METHODS

We used 10 domestic pigs of Danish Landrace/Duroc/Hampshire/Yorkshire breed (aged 3–4 months; weight, 28–38 kg). The Danish Animal Experiments Inspectorate granted permission for the use of the animals, and the experiments were conducted in compliance with the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research.

Anesthesia and Animal Preparation

The pigs were prepared for experiments as previously described.1 Briefly, sedation was induced by intramuscular injection of a mixture of tranquillizers (midazolam, zolazepam, tiletamin, zylazine, ketamine, and methadone), followed by pentobarbital (Mebumal; SAD Copenhagen, Denmark) into the ear vein. After intubation, the animals were artificially ventilated and catherized into the femoral artery and vein, and the superficial epigastric vein. During the oxygen measurements, anesthesia was maintained by continuous administration of pentobarbital in one vein, and fentanyl (Haldid; Janssen-Cilag, Birkerode) and pancuronium bromide (Pavulon; Organon, Oss, the Netherlands) in the other vein. During the BRVO induction procedure in the long-term experiments, propofol (Propofol; Abbott, Gentofte, Denmark) was used as the only anesthetic. Propofol has recently been shown to cause

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NO-mediated vasodilatation and modulate inner retinal function. Therefore, we used only this drug during the BRVO induction procedure in the long-term experiments, and no measurements were obtained until 1 week after the anesthesia.

A pressure transducer was connected to the arterial catheter for continuous measurements of arterial blood pressure (MAP). Heart rate (HR) was recorded from the ECG electrodes placed on the animal. MAP, ECG, and rectal temperature were sampled continuously. The pig was placed in a sling and the head was secured in a stereotactic headholder to avoid movements of the eye. A speculum was placed between the eyelids. All experiments were performed on the right eye. The animals were ventilated at approximately 4 L/min, with a continuous flow of 21% O2-79% N2O from a variable-volume respirator. Arterial gas samples were analyzed for arterial PO2 (aPO2), arterial PCO2 (aPCO2), and arterial pH (apH; ABL 605, blood gas analyzer; Radiometer, Copenhagen, Denmark). Before the administration of dorzolamide, arterial aPO2, aPCO2, and apH were kept within normal levels by adjusting the rate of ventilation.

The pupil was dilated and anesthetized with topical 1% tropicamide (Mydriacyl; Alcon, Rødovre), 2.5% methaoxedrine (Metaoxedrin; SAD, Copenhagen, Denmark), and 0.4% oxybuprocaine (Oxybuprokain; SAD; Copenhagen, Denmark) as eye drops. A sclerotomy was placed 2.0 mm behind the corneal limbus in the superior nasal quadrant, and a plastic cannula (16-gauge) was placed in the sclerotomy. Dorzolamide hydrochloride (Merck, Sharp & Dohme, Glostrup, Denmark) was dissolved as a 3% solution in 100 mM citrate buffer (pH 5.6).

**Induction of BRVO**

BRVO was induced by a blunt diathermy needle inserted into the vitreous cavity through a sclerotomy 2 mm behind the limbus. The branch retinal venule was gently compressed for 5 seconds to empty the vein to prevent post-BRVO hemorrhaging. Diathermy was applied for 5 to 7 seconds on the superior vein half a disc diameter away from the disc margin (Fig. 1). The outcome was a white lesion completely covering the venule, and a visible congestion inside the venule peripheral to the occlusion. In the acute experiments, oxygen measurements were performed with an oxygen-sensitive electrode, before and after BRVO induction. Great care was taken to avoid loss of vitreous during the replacement of the diathermia probe and oxygen electrode. To maintain a stable IOP, a second sclerotomy was connected to a reservoir containing Ringer’s lactate solution during the BRVO induction procedures. No vitrectomy was performed, and only a negligible amount of vitreous was lost and replaced by the Ringer’s lactated solution during the experiments. Room-air–equilibrated Ringer’s lactate solution has an oxygen tension of 21 kPa and may be expected to influence the oxygen tension in the preretinal vitreous; 10 mL of room air-equilibrated Ringer’s lactate will increase the average porcine vitreous oxygen tension by 0.07 kPa.

The same surgeon (ESc) performed all BRVO procedures. In selected cases, fluorescein angiography was performed to visualize the effect of the occlusion during the first week after the BRVO (Figs. 1, 2).

**FIGURE 1.** Color fundus (CF) photograph and fluorescein angiogram (FA) of the same retina 1 hour after BRVO induction: **Left**: the position of the applied BRVO (✱), and the three oxygen measurement positions: I is the BRVO-affected area, and N1 and N2 are the two control positions, which are situated two optic disc diameters away from the optic disc, and therefore are not directly visible. Distal to the occlusion, the affected retinal venule is dark and dilated; between the occlusion and the optic disc, it is hardly visible. In the FA, there is delayed filling of the occluded venule. There is a clear watershed zone between the BRVO-affected area and the nonischemic area, and small vessels and capillaries are more visible in the unaffected areas than in the BRVO-affected area.

**FIGURE 2.** Color fundus (CF) photograph and fluorescein angiography (FA) of the same retina as in Figure 1, 1 week after BRVO induction. **Left**: CF photograph showing the BRVO-affected area, characterized by localized retinal hemorrhages and edema. **Right**: FA of the same retina, showing delayed filling of the superior retinal vein.
Oxygen Measurements

We used a Clark-type polarographic oxygen-sensitive electrode to measure the oxygen tension in the preretinal vitreous, as previously described. Briefly, the platinum electrode was mounted with an internal Ag/AgCl reference electrode inside a 20-gauge needle (model 768 20R; Diamond General, Ann Arbor, MI). The tip of the needle was sharpened at the end to an area of 0.15 mm² in a crescent shape. The signal from the electrode was measured continuously with a chemical microsensor (model 1231; Diamond General). The electrode was advanced by a micromanipulator through a Teflon cannula inserted in the sclerotomy. The positioning of the electrode was guided by indirect ophthalmoscopy, and the tip of the electrode was placed 0.5 mm above the retina in three different positions: One position above the center of the ischemic area, and two positions above the retinal area which was not affected by the BRVO (see Fig. 1). The electrode was always placed in retinal areas without any visible retinal vessels, and at a distance of two optic disc diameters away from the optic disc. Several measurements were repeated to confirm the reliability of the electrode positioning and the oxygen recording system. Each oxygen tension measurement was preceded by 4 to 10 minutes of baseline recording. The entry sites of the sclerotomy were watertight, and all eyes maintained normal shape throughout the experiments. The intraocular pressure was not measured.

The oxygen electrode was calibrated before and after each experiment in 100% N₂ and 5% O₂-95% N₂ in a calibration cell (model 1251; Diamond General Development). The drift of the oxygen electrode was less than 0.1 kPa per hour.

Experimental Protocol

Two types of experiments were performed. In the first group of five pigs, we investigated the acute effects of the BRVO induction and the effects of systemic dorzolamide in this setting. In this group, oxygen measurements were obtained over control retinas and BRVO-affected retina at baseline (before BRVO induction), 1 hour after BRVO induction, 3 hours after BRVO induction, and 30 minutes after intravenous administration of 500 mg dorzolamide (3-4 hours after BRVO induction). One and 3 hours after BRVO induction, RPO₂ was significantly decreased by 0.67 ± 0.29 kPa (P < 0.001, critical value = 0.013, n = 5) and 0.94 ± 0.13 kPa (P < 0.001, critical value = 0.009, n = 5), in the BRVO-affected area. In the control areas (non-BRVO areas), the RPO₂ increased significantly (by 0.51 ± 0.14 kPa; P < 0.001, critical value = 0.017, n = 5) 1 hour after BRVO induction, but had returned to normal values, 2.82 ± 0.17 kPa (mean ± SD, n = 4) 3 hours after BRVO induction.

Approximately 3 hours after induction of BRVO, 500 mg dorzolamide was administered intravenously. The effects of dorzolamide on RPO₂ were recorded 30 minutes later. Dorzolamide caused a significant increase in RPO₂ of 1.00 ± 0.17 kPa (P < 0.001, critical value = 0.05, n = 3) in the control areas (non-BRVO areas). In the BRVO-affected retina, dorzolamide caused a significant increase in RPO₂ of 0.36 ± 0.21 kPa (P = 0.015, critical value 0.025, n = 5).

Effect of 1-Week-Old BRVO and Dorzolamide

Figure 4 shows the RPO₂ measured in five pigs 1 week after the experimental BRVO. In the BRVO-affected areas, RPO₂ was 0.67 ± 0.29 kPa (P < 0.01, critical value = 0.025, n = 5) below the level in the control areas (non-BRVO areas), where RPO₂ was 2.35 ± 0.37 kPa.

One hour after intravenous administration of 500 mg dorzolamide, RPO₂ had increased significantly (by 0.67 ± 0.40 kPa; P < 0.01, critical value = 0.017, n = 5) over the BRVO-
affected areas to 2.36 ± 0.85 kPa, whereas the increase was 1.08 ± 0.56 kPa over the control areas (P < 0.001, critical value = 0.013, n = 5). After dorzolamide, the RPO₂ levels in the BRVO-affected areas were not different from the RPO₂ measured in the control areas before BRVO induction (P = 0.9, critical value = 0.5, n = 5). This means that dorzolamide in average had restored normal oxygen tension in the BRVO-affected areas (Fig. 4).

Figure 5 shows the differences in RPO₂ between the control areas and the BRVO-affected areas, 1 hour, 3 hours BRVO, and 1 week after BRVO. The hypoxia remained at a nearly constant level at 1 and 3 hours after BRVO, whereas it decreased by 42% 1 week after the BRVO induction.

Figure 6 shows typical recordings of the RPO₂ in the BRVO-affected areas after administration of 500 mg dorzolamide, at 3 to 4 hours and 1 week after BRVO.

Relationship between Blood PCO₂ and RPO₂
Table 1 shows values of arterial pH, PCO₂ and PO₂, both before and after induction of BRVO and after administration of dorzolamide. As expected, the induction of BRVO did not affect these values, but dorzolamide did decrease arterial pH and increased arterial PCO₂ significantly in both short- and long-term experiments. These results are consistent with previous findings in our group.²,¹⁹ Hypercapnia has been shown to cause optic nerve head and retinal vasodilation.⁶,²⁰ Figure 7 shows the ratio between the dorzolamide-induced changes in RPO₂ and ΔPCO₂, ΔRPO₂/ΔPCO₂, after acute BRVO, and 1 week later. After acute BRVO, the ratio ΔRPO₂/ΔPCO₂ was significantly lower in BRVO-affected retina when compared with control retina (unpaired t-test, P < 0.05). One week later, there was no significant difference in this ratio in the BRVO-affected retinas and control retinas (unpaired t-test, P = 0.2).

DISCUSSION
Our model of ischemic BRVO in pigs displayed the characteristic features of BRVO on color fundus photography and fluorescein angiography, and these findings are in accordance with previous reports.⁷ We found that the areas affected by BRVO were hypoxic, with a 39% reduction in RPO₂ 3 hours after the BRVO, and a 30% reduction 1 week later. This level of hypoxia resembles what previously has been reported in minipigs, where argon laser photocoagulation was used to induce BRVO.⁶,⁷ BRVO induced by diathermia has produced somewhat higher levels of acute ischemia, 70% in cats.⁵ We found that the hypoxia induced by BRVO decreased within the first week (Fig. 5), perhaps due to formation of shunt vessels toward nonischemic areas as well as some degree of recanalization of the occluded vein, as indicated elsewhere.²¹

We have reported that intravenous dorzolamide (500 mg) increases RPO₂ by 11% and preoptic nerve oxygen tension (ONPO₂), by 20% to 52%.¹–³,¹⁹ In the present study, we found that 500 mg intravenous dorzolamide causes a similar increase in RPO₂ in the normal retina, both 3 to 4 hours after BRVO and 1 week later. In the acutely BRVO-affected retina, dorzolamide significantly increased RPO₂ by 20% 3 to 4 hours after the BRVO induction.

In a previous study in minipigs, where RPO₂ was measured with a 10-μm oxygen electrode, 500 mg intravenous acetazolamide did not increase RPO₂ significantly in a retina affected by acute BRVO.⁶ However, carbogen breathing, either alone or in combination with acetazolamide, significantly increased the oxygen tension in acute BRVO-affected retinal areas in this...
study. Also, carbogen breathing and acetazolamide had additive effects on oxygen tension in the optic nerve head and in retinal areas affected by acute BRVO. The differences in methodology and experimental animals and the fact that dorzolamide has been demonstrated to be more potent than acetazolamide may explain why we found a significant effect after dorzolamide, as opposed to the findings in the previous study on acetazolamide.

The effects of carbonic anhydrase inhibition have not been studied beyond the immediate post-BRVO period, when the effects of retinal ischemia are confounded with the effects of the surgical trauma and inflammation, as well as secondary arteriolar vasoconstriction. We found that 1 week after BRVO induction, dorzolamide increased RPO2 over BRVO-affected retina by 40% and that this in effect restored normal oxygen tension in the BRVO-affected areas (Figs. 4, 7).

The increase in RPO2 after carbonic anhydrase inhibition (dorzolamide) must be due to arteriolar vasodilation in the retinal circulation. Although choroidal blood flow has been shown to be increased in patients with BRVO, dorzolamide has been found to be independent of the choroidal blood flow, except when an ischemic retina is subjected to systemic hypoxia. Hence, carbonic anhydrase inhibition with dorzolamide also seems to induce retinal vasodilatation and increased blood flow in ischemic retina, especially when the confounding effects of acute surgical trauma has waned.

It is well known that dorzolamide causes an increase in aPCO2, and that CO2-induced vasodilation may be part of the mechanism behind the increase in RPO2 caused by the drug. In Figure 7, the dorzolamide-induced RPO2 response was normalized to the dorzolamide-induced increase in aPCO2. It shows that in areas affected by acute BRVO, the ∆RPO2/∆aPCO2 ratio was significantly smaller than in control areas. However, 1 week later, the BRVO-affected areas regained their responsiveness to CO2, and there was no longer any significant difference between the ∆RPO2/∆aPCO2 ratio obtained over control retina and over BRVO-affected retina. This finding may be interpreted as if the microcirculation in the BRVO-affected area recovered from the acute trauma and regained responsiveness to hypercapnia during the first postoperative week.

It should be noted, however, that the CO2 tension in the arterial blood did not reflect the CO2 tension in the retinal microcirculation, particularly in areas affected by BRVO. Also, the experimental model of sudden, complete occlusion does not emulate the progressive retinal ischemic diseases encountered in humans.

Our measurements of RPO2 reflect the oxygen supply at the surface of the retina. Whether dorzolamide is capable of normalizing intraretinal oxygen supply in retinal ischemia remains to be shown. Another important question is whether the effect is durable or subject to tachyphylaxis. Isovolumetric hemodilution is sometimes used in the human clinic to increase oxygenation in ischemic retina. It might be interesting to test the effect of this modality in combination with carbonic anhydrase inhibition in experimental BRVO.

In the experiments with acute BRVO, we observed an increase in RPO2 1 hour after BRVO induction. Retinal trauma and inflammation are well known triggers of NO induced retinal vasodilation. The acute surgical trauma is a likely explanation for the observed increase in RPO2. On the other hand, we maintained the intraocular pressure during the diathermia procedure by an infusion cannula connected to a Ringer’s lactate reservoir. This method may have caused influx of room-air-equilibrated Ringer’s lactate into the anterior vitreous, which in turn may have diffused through the vitreous to the electrode. As mentioned earlier, no vitrectomy was performed, and the amount of fluid entering the eye was minute. Nevertheless, this artifact may have caused the temporary increase in RPO2 after the BRVO induction to be erroneously high in the control areas. However, it should be noted that this artifact affects the control retina in the same way as the BRVO-affected retina.

The vitreous is firmly attached to the retina in the young pigs used in the present study, and oxygen transport in the posterior vitreous can be assumed to be entirely diffusive. We measured the oxygen tension in the vitreous, 0.5 mm from the retinal surface. The electrode tip was placed 3 to 8 mm from the border between normal and BRVO-affected retina. Accordingly, the lateral diffusion distance from the electrode to either normal or BRVO-affected retina is larger than nearly an order of magnitude than the diffusion distance from the retina to the vitreous.

### Table 1. Arterial Blood Gas Levels

<table>
<thead>
<tr>
<th></th>
<th>aPCO2</th>
<th>aPO2</th>
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<tr>
<td>Before BRVO</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 h after BRVO</td>
<td>6.51 ± 0.23</td>
<td>18.0 ± 3.7</td>
</tr>
<tr>
<td>3 h after BRVO</td>
<td>6.77 ± 0.45</td>
<td>15.5 ± 5.9</td>
</tr>
<tr>
<td>After dorzolamide</td>
<td>8.65 ± 0.53</td>
<td>15.6 ± 2.7</td>
</tr>
<tr>
<td>aPCO2</td>
<td>8.35 ± 0.43*</td>
<td>16.7 ± 2.5</td>
</tr>
<tr>
<td>aPO2</td>
<td>6.82 ± 0.68*</td>
<td>15.5 ± 2.4</td>
</tr>
</tbody>
</table>

n = 5.

*P < 0.01, by paired t-test.
electrode tip. Hence, we find it unlikely that our results are confounded by lateral diffusion of oxygen from adjacent non- BRVO areas, as oxygen measurements close to the retina reflect the nearby intraretinal oxygen tension. 7,24,29 Although a smaller electrode was used, this caveat also holds for the previous study on acetazolamide. 21 It is a clear advantage of smaller electrode was used, this caveat also holds for the preretinal measurements that they do not cause trauma to the retina, in contrast to intraretinal measurements. The eventual relevance of the present findings to human disease remains to be determined.

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