Impaired Hyaloidal Circulation Function and Uncoordinated Ocular Growth Patterns in Experimental Retinopathy of Prematurity

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PURPOSE. To test the hypotheses that, in the newborn rat model of retinopathy of prematurity (ROP), the hyaloidal circulation is functionally impaired and its development is not well coordinated with that of other ocular structures.

METHODS. The functional response of the hyaloidal circulation to a carbogen inhalation challenge was noninvasively evaluated using magnetic resonance imaging (MRI) in day 12 rats raised under either variable oxygen conditions (experimental ROP, n = 8) or room air (control, n = 8). A similar MRI examination was performed in separate experiments using either day 18 newborn control rats (n = 3) or adult rats (n = 9). For each experiment, the hyaloidal circulation perfusion response to carbogen, the functional spatial extent of the hyaloidal circulation in vitreous, and the volumes of vitreous and lens were estimated from MRI enhancement maps.

RESULTS. The hyaloidal perfusion response to carbogen breathing in the newborn rats decreased as follows: control day 12 > experimental day 12 > control day 18; no measurable hyaloidal function was found in the adult rat. Regression analysis indicated a relatively poorer superior-inferior correlation in the temporal response to carbogen inhalation for the experimental animals than in the control newborn rats. The vitreous volume decreased in control rats as expected (adult rat > day 18 > day 12). Good agreement was found between the MRI-determined adult rat vitreous volume (56 ± 2 µl) and that of previous reports. Functional hyaloidal volumes during carbogen breathing were not significantly different (P > 0.05) between day 18, day 12 control, and experimental newborn rats. The ratio of this functional hyaloidal circulation extent volume to vitreous volume was significantly different (P < 0.05) between these groups. Covariance analysis revealed a relatively less coordinated development between the functional hyaloidal volume and the vitreous volume in experimental animals than in age-matched control animals, whereas there was coordinated evolution of the hyaloidal circulation and the lens in all the animals.

CONCLUSIONS. Carbogen-enhanced MRI appears to be a powerful new and noninvasive approach for assessing the functionality of the hyaloidal circulation (that is, its ability to respond to a carbogen challenge) and quantitatively comparing the functional hyaloidal extent to other ocular volumes in the same eye during development and during the disease process. Evidence is presented here for the first time that supports the authors’ hypotheses that the function of the hyaloidal circulation in experimental ROP is impaired and that the growth of ocular components are less coordinated.


Retinopathy of prematurity (ROP) is a major cause of blindness and vision loss in low-birth-weight infants.¹ Although the pathogenesis of ROP is unknown, its blinding complications are associated with retinal vessel hemo-

dynamic abnormalities, such as an attenuation of retinal blood vessels and angiogenesis.²³ Because the retinal and hyaloidal circulations share the central retinal artery, it is expected that the hyaloidal system also exhibits some functional hemodynamic disease. The hyaloidal circulation is a transitory network of blood vessels responsible for nurturing the embryonic and fetal lens.⁴ Regression of this circulation is complete by approximately the seventh month of pregnancy in humans and the third to forth weeks after birth in the rat.⁵⁶ Current methods provide little quantitative information about the hemodynamic function of the hyaloidal circulation in vivo or its relationship to other ocular components.

To understand better the ocular complications associated with ROP, a newborn rat model of ROP has been developed, that closely mirrors many aspects of the human condition.⁷ In the rat model, newborn animals are raised for 2 weeks in a variable oxygen environment designed to provide the animals with clinically relevant blood oxygen levels.⁸ At 2 weeks there is consistent attenuation of retinal blood vessels. On removal to room air, angiogenesis is produced during the next 6 days in
100% of the eyes in this model. For the present study, another important feature of this model is that it displays persistent, enlarged, and tortuous hyaloidal vessels at 2 weeks. Aside from the identification of this condition, no quantitative functional information concerning the hyaloidal circulation, its connection with disease outcome, or its relationship to other ocular structures is available.

Recently, a novel and noninvasive method for studying the hyaloidal circulation was discovered during magnetic resonance imaging (MRI) of the newborn rat breathing oxygen-enriched gases (for example, carbogen [95% O₂/5% CO₂]). In these experiments, the profile of the hyaloidal circulation could be readily distinguished from the surrounding vitreous as a result of significant increases in the MRI signal intensity that occurs during carbogen inhalation; the hyaloidal circulation was not observed during room air breathing. Because these signal changes were likely produced by a combination of a rise in oxygen concentration and an increase in hyaloidal blood perfusion (vide infra), they represent the functional ability of the hyaloidal circulation to respond to the carbogen gas. In addition, the resultant signal enhancement provided a unique visualization of the “functional” extent of hyaloidal circulation and an opportunity to compare it to the dimensions of other ocular structures. In contrast to fluorescein angiograms, this net functional response of the hyaloidal vasculature during carbogen breathing does not contain details about individual hyaloidal vessels. Instead the MRI method measures the sagittal cross-sectional extent of the hyaloidal profile during carbogen breathing and yields an estimate of the functional envelope volume.

In this study, carbogen-enhanced MRI was used to examine the hyaloid’s perfusion response to carbogen and the resultant functional extent of the hyaloidal circulation in the vitreous in the experimental model of ROP and age-matched control newborn animals. To examine the sensitivity of this method, the hyaloidal circulation’s response to carbogen breathing was also studied in day 18 newborn control rats. At this age, the hyaloidal circulation has significantly regressed. In addition, ocular volumes from the lens and vitreous were determined. To evaluate the accuracy of the MRI volume estimate, the vitreous volume in the adult rat was measured and compared with that found in the literature.

**METHODS**

In all cases, the animals (ROP day 12 newborn rats [n = 8], age-matched control newborn rats [n = 8], day 18 control newborn rats [n = 3], and adult rats [n = 9]) were treated in accordance with the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research, the National Institutes of Health Guide for the Care and Use of Laboratory Animals, and institutional (University of Texas Southwestern Medical Center) guidelines on the use of animals in research. The newborn rat model of ROP has been described in detail elsewhere. Briefly, Sprague-Dawley mothers and litters were housed in a modified pediatric incubator, in which the oxygen levels were varied in a step fashion between 50% and 100% every 24 hours for the first 12 days after birth. A second group of mothers and age-matched litters was maintained in room air. On the day of the experiment, animals were removed from the incubators and flown from Arkansas to Texas. MRI experiments, as previously described, were performed approximately 5 to 6 hours after the animals were removed from the incubators. The last animal examined was studied approximately 14 hours after its removal from the incubators. This was approximately 1.5 days before histologic evidence of neovascularization. Although some associated retinal hemodynamic changes in advance of angiogenesis may have been produced during this time, the similar scatter, for controls and ROP groups in the hyaloidal circulation’s mean signal intensities (see Fig. 2) and the estimated ocular volumes (see Table 1), suggests that this was not a confounding factor for these experiments.

Anesthesia was induced by an intraperitoneal injection of urethane (1.5 g/kg, 36% solution, made fresh each day). Each rat was gently positioned on an MRI-compatible homemade holder with its nose placed in a plastic nose cone. Animals were allowed to breathe spontaneously during the experiment. To maintain the rats’ core temperatures, a recirculating warm-water blanket was used. Rectal temperature, pulse, and hemoglobin oxygen saturation were continuously measured inside the magnet. The MRI experiments were performed as previously described. MRI data were acquired on a 4.7-T system using a two-turn, transmit-receive surface coil (1.5-cm diameter) placed over the eye and an adiabatic, spin-echo-imaging sequence (repetition time, TR 1 second; echo time, TE 18 msec; number of acquisitions, NA 1; matrix size, 128 X 256; slice thickness, 0.8 mm; field of view, 45 mm X 45 mm for adult rats and 28 mm X 28 mm for the newborn ones; acquisition time, 2 minutes per image). Care was taken to ensure that the imaged slice contained the plane of symmetry of the eye. A capillary tube (1.5-mm inner diameter) filled with distilled water was used as the external standard. Ten sequential 2-minute images were acquired as follows: six control images while the animal breathed room air and four images during 8 minutes of carbogen breathing. Carbogen exposure was started immediately at the end of the sixth image. A third group (n = 3) of room air control rats was studied at day 18 after birth, and a set (n = 9) of adult rats was studied after the experimental MRI protocol outlined above.

Changes in the MRI signal intensity produced during the 8 minutes of carbogen breathing were used noninvasively to map the hyaloid’s signal enhancement on a pixel-by-pixel basis and its evolution during carbogen breathing as previously described. As a measure of the perfusion response index to carbogen breathing, the mean MRI enhancement signal intensities (in arbitrary units normalized to the external water standard) and standard error were calculated for the hyaloidal silhouettes in all the newborn animals. The edge of the functional signal intensity increase of the hyaloidal circulation was not well defined, so regions of interests were chosen in a subjective manner based on extensive examination of the time series data in cine loops. To compensate for this potential bias, the average of two masked investigators is presented. The progression of the perfusion response index was monitored by plotting the superior hemiocular MRI-enhanced intensities from the time sequence obtained during carbogen breathing against the inferior ones and calculating the regression coefficient for each set of newborn animals. In this case, the hemiocular regions had clearly identifiable boundaries of the lens and the retina-choroid complex and were not considered to have a subjective bias. Ocular volumes (lens, vitreous, and hyaloid) were estimated from the pixel-by-pixel maps using the Pappus theorem for the special case when the volume sought has cylindrical symmetry near an axis contained in the symmetry plane. In this case, the volume is equal to half the area of the...
cross-section near the symmetry plane, multiplied by $2\pi r$, where $r$ is the distance between the cross-sectional centroid and the symmetry axis (Fig. 1). The two key assumptions for the application of the Pappus theorem to the MRI images are that the MRI slice contains the plane of symmetry of the eye and that the ocular features have cylindrical symmetry near an axis contained in their imaged plane. The good agreement between the MRI vitreous volumes and those in the literature for the adult rat supports the reasonableness of these assumptions (vide infra). The second assumption seems reasonable for the functional extent of the hyaloidal circulation of the control group but may not be as sound for the ROP group (vide infra). An attempt was made to compensate for this by averaging superior and inferior hemifunctional hyaloidal extent volumes.

For the day 12 newborn animals, the data were compared using the two-tailed Student's unpaired $t$-test to assess significance ($P < 0.05$). To determine a possible correlation between the hyaloid, vitreous, and lens volumes, covariance analysis of the data was performed and regression coefficients were calculated.

**RESULTS**

**Perfusion Response to Carbogen Breathing**

Figure 2 shows the mean hyaloidal signal intensity changes in response to the carbogen inhalation for both 12- and 18-day newborn animals. The mean intensities in the newborn rats decreased significantly ($P < 0.05$) between groups as follows: day 12 controls > day 12 ROP > day 18 controls. As expected, no measurable hyaloidal function was found in the adult rat.

**Variations in the Hyaloidal Perfusion Response**

To evaluate perfusion progression differences, the correlation between superior and inferior hemiocular signal enhancements from the four images obtained during carbogen breathing for all animals in each group (day 12 control and ROP newborn rats, Figs. 3A, 3B) was investigated. The linear regression coefficient values were 0.70 and 0.52 for the control and experimental groups, respectively. A similar analysis of the day 18 newborn rats yielded a regression coefficient of 0.98.

**Ocular Volumes**

Table 1 summarizes the calculated values obtained for the lens, vitreous, and hyaloidal circulation volumes in control and experimental newborn rats and in adult rats. No significant differences ($P < 0.05$) were found in the functional hyaloidal extent in response to carbogen breathing among the groups. The vitreous volumes decreased as follows: adult rat > day 18 control > day 12 control > day 12 ROP. Figure 4 is a plot of the mean ratio of the functional hyaloidal circulation volume to vitreous volume and standard error of the mean for the experimental and control newborn rats. A significant difference ($P = 0.002$) between the ratios of the two groups was found. To highlight the variations observed, the hyaloidal silhouettes are presented for day 12 experimental and control newborn rats from the MRI images (Fig. 5).

**DISCUSSION**

In this study, the hyaloidal vascular system was manifested and studied using carbogen-enhanced MRI. The resultant increased

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**TABLE 1. Average Ocular Volumes (mean ± SEM)**

<table>
<thead>
<tr>
<th></th>
<th>Lens ($\mu l$)</th>
<th>Hyaloidal Circulation ($\mu l$)</th>
<th>Vitreous ($\mu l$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adult rat</td>
<td>7.3 ± 0.6</td>
<td>4.8 ± 0.4</td>
<td>56.5 ± 1.8</td>
</tr>
<tr>
<td>Control day 12 newborn rat</td>
<td>7.1 ± 0.5</td>
<td>4.9 ± 0.3</td>
<td>18.0 ± 1.2</td>
</tr>
<tr>
<td>ROP day 12 newborn rat</td>
<td>13.0 ± 0.8</td>
<td>4.1 ± 0.1</td>
<td>10.7 ± 1.1</td>
</tr>
<tr>
<td>Control day 18 newborn rat</td>
<td>13.0 ± 0.8</td>
<td>4.1 ± 0.1</td>
<td>10.7 ± 1.1</td>
</tr>
</tbody>
</table>

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**FIGURE 1.** Schematic representation of the application of the Pappus theorem. The special case is when the object has cylindrical symmetry near an axis contained on its cross-sectional plane (for example, a hemiocular globe). $A =$ area of half the cross-section (shaded region), $r =$ distance between the centroid in $A$ and the symmetry axis. The volume is $V = 2\pi rA$.

**FIGURE 2.** Magnetic resonance imaging intensity change of the hyaloidal circulation during 8 minutes of carbogen breathing (mean ± SEM). The mean intensities decrease ($P < 0.05$) as follows: controls day 12 > ROP day 12 > controls day 18.
FIGURE 3. Superior hemiocular magnetic resonance imaging (MRI) intensity versus inferior hemiocular MRI intensity in day 12 controls (A) and ROP animals (B). The solid lines represent the best fit to a linear equation: A: $y = -1.012 + 0.8886x$, $r = 0.701$; B: $y = 17.135 + 0.4022x$, $r = 0.517$; where $y$ is superior hemiocular enhancement, $x$ is inferior hemiocular enhancement, and $r$ is correlation), and the dashed lines represent the 95% confidence intervals.

Hyaloidal intensity was likely produced by a combination, of a rise in oxygen concentration and an increase in hyaloidal blood perfusion, which resulted in the so-called in-flow effect. The in-flow effect is a change in MRI signal intensity resulting from a change in the delivery of fresh MRI signal into the slice (along with a change in the removal of saturated MRI signal). These MRI signal intensity changes constitute a measure of the hyaloidal perfusion response to the carbogen challenge.

One of the key findings of this investigation is that day 12 ROP rats exhibited a significantly lower mean signal intensity change (73.2) than day 12 control rats (98.3) (Fig. 2). A direct comparison of this result with findings in the literature is not possible because currently available methods have not assessed hyaloidal function in vivo during an inhalation challenge. Nonetheless, a previous fluorescein angiography study in a similar newborn rat model of ROP did show a relatively increased vascular diameter of the hyaloidal vessels in day 14 experimental animals compared with controls. We speculate that such enlarged vessels will have a decreased perfusion response to carbogen inhalation. To further evaluate the sensitivity of this MRI measurement, day 18 control animals, which have developmentally regressed vessels, were examined. In these animals, the MRI signal intensity changes during carbogen were significantly lower (P = 0.0003) than for day 12 control animals, consistent with a decreased perfusion response. Thus, although detail about individual hyaloidal vessels cannot be determined from the MRI experiment, as they can be from fluorescein angiograms, the net functional response of the hyaloidal vasculature may be studied, and this is expected to have practical diagnostic and prognostic value. These results also support our hypothesis that the functional ability of the hyaloidal system is relatively decreased in the experimental newborn rat model of ROP.

Next, the progression of the signal intensity changes during carbogen inhalation in the superior and inferior hemiocular sections were examined for each group with regression analysis (Figs. 3A, 3B). The resultant linear correlation coefficient provided a measure of symmetry in the progression of the perfusion changes (that is, the more symmetric the progression, the closer the regression coefficient will be to 1). Note that oxygen diffusion mechanisms contribute in a similar fashion in both eye hemispheres and therefore are not expected to...
FIGURE 5. Hyaloidal silhouettes. The two columns on the left correspond to the hyaloidal functional silhouette produced by averaging during 8 minutes of carbogen breathing in day 12 control animals; the two columns on the right correspond to the day 12 retinopathy of prematurity (ROP) group. The outline of the retina-choroid is also presented for anatomic reference. Note the more irregular appearance of the hyaloidal shadow and the smaller vitreous volume in the ROP animals than those of the controls.

In general, the day 12 and day 18 control animals showed better correlation (regression coefficients of 0.70 and 0.98, respectively) than did the experimental group (0.52). We speculate that the relatively greater asymmetric progression in the perfusion of the hyaloidal circulation of the ROP animals is a result of the perfusion proceeding through a more tortuous path. This interpretation is consistent with the more irregular hyaloidal profiles observed for this group when compared with age-matched control rats (Fig. 5). Further experiments are planned to clarify this interpretation. The relatively greater asymmetry in the progression of the hyaloidal perfusion response in the ROP animals is consistent with a more impaired hemodynamic functionality in these animals than in age-matched controls.

One possible alternative explanation for the apparent abnormal function of the hyaloidal hemodynamics in the ROP animals may be systemic physiological differences between groups during carbogen breathing. Previous analysis of the blood gas values for each treatment group revealed similar PaO₂ and PaCO₂ values during carbogen breathing. A significant relative acidosis was found in the ROP group. Two mechanisms by which acidosis could introduce error into the interpretation of the MRI measurement are altering vasoactivity so that a smaller than expected response to carbogen breathing is produced and shifting the hemoglobin oxygen saturation curve to the right (Bohr effect). However, a change in blood pH per se is not known to elicit a vasomotor reaction from retinal vessels or to affect the regulation of vascular tone. In addition, a Bohr effect would favor oxygen off-loading to the tissues, which tends to raise the level of oxygenation and thereby to produce an increased MRI signal that would be in opposition to our current observation. Furthermore, the significant asymmetric response within the ROP group could not be a result of systemic reasons. Thus, the decreased hyaloidal perfusion response to carbogen inhalation in the ROP animals is consistent with local, not systemic, differences in perfusion.

In addition to this functional information, the silhouette produced during carbogen breathing provided a noninvasive visualization of the functional cross-sectional extent, or envelope, of the hyaloidal circulation in the vitreous. The principal advantages of this measurement are that it allows for quantitative comparisons of the hyaloid's functional extent with other ocular volumes and that it may provide new insights into normal and pathologic ocular growth patterns. Application of the Pappus theorem to single-slice MRI images to estimate ocular volumes was validated using adult rat eye measurements. Reasonable agreement was found between the MRI-determined adult rat vitreous volume (56.5±1.8 μl, mean ± SEM) and that measured in the literature (56.9±5.2 μl, mean ± SD). Furthermore, good agreement was also found by comparing the vitreous volumes estimated from published schematics of the adult rat eye (58 μl and 55 μl) to the MRI data.

In this study, the functional hyaloidal volume measured by the MRI experiment was not significantly different between experimental and age-matched control animals (Table 1). This result appears to conflict with the results of a previous fluorescein angiography study in a similar newborn rat model, which found enlarged hyaloidal vessels and an increased vessel density for day 14 ROP animals, compared with controls. The fluorescein angiography experiment is typically used to observe single vessels during room air breathing, whereas the MRI method measures the sagittal cross-sectional extent of the hyaloidal profile during carbogen breathing (that is, the functional hyaloidal volume between the optic nerve and the full span of the lens). Thus, the MRI and the fluorescein experiments produce different views of the hyaloidal circulation, namely a functional envelope volume versus individual vessels, respectively. Therefore, the results from these two experiments cannot accurately be compared, because they reveal different aspects of the circulation. However, the functional response to carbogen breathing, not functional extent, can be compared with previous fluorescein angiography data, as discussed above, and the two experiments do produce apparently consistent results. That is, fluorescein angiography has demonstrated that the hyaloidal circulation in this model consists of enlarged vessels. These vessels may be unable to respond with more dilation during carbogen breathing and therefore would be expected to produce a lower MRI signal intensity change.

To examine this result further, the lenticular volumes in each group were analyzed because the hyaloidal circulation is thought to be important as a lens vascular bed during development. No significant difference in lens volume was found (P = 0.92) between day 12 control and ROP animals. This result is consistent with our present observation that a similar functional hyaloidal circulation volume exists between these groups of newborns and that it is supported by previous electron microscope study results that demonstrated that the hyaloidal circulation extends between the optic nerve and the full span of the lens in age-matched control and oxygen-exposed animals. A comparison with the day 18 animals is not included, because it is expected that the relative sizes of
different ocular features at this developmental stage will have changed with respect to day 12, as the hyaloidal circulation becomes atrophied and the lens and vitreous develop further.

The relationship between the functional hyaloidal volume and the vitreous volume during development and disease was also examined. Comparing the two control groups (day 12 and 18) first to investigate developmental differences in the regression of the hyaloidal circulation with age revealed a significantly smaller ratio of functional hyaloidal circulation volume to vitreous volume for day 18 (0.14 ± 0.02, mean ± SEM, \( P = 0.011 \)) than in day 12 control animals. This was primarily a result of a substantial increase in vitreous volume in day 18 animals (Table 1). Comparison of this ratio for day 12 control and ROP animals showed a statistically significant (\( P < 0.05 \)) ratio for the ROP animals (0.495 versus 0.273). This was a result, again, of the relatively smaller vitreous volumes of the ROP animals. The significant difference (\( P = 0.006 \)) in vitreous volumes likely reflected the significant discrepancy in weight between groups (11.6 ± 0.7 g for ROP versus 22.1 ± 1.5 g for control; mean ± SEM, \( P = 0.0001 \)). To the best of our knowledge, the ratio of functional hyaloidal extent volume to vitreous volume has not been examined in the newborn rat model of ROP. This ratio appears to be a sensitive parameter for investigating development and pathologic changes in growth patterns in this model, and it is worth further investigation.

Close inspection of this ratio in Figure 4 revealed relatively larger error bars for the ROP animals than for the control animals. To understand this better, covariance analysis was performed among the hyaloidal, lens, and vitreous volumes. Evidence for a coordinated development between the hyaloidal circulation and lens was found based on a positive regression coefficient (0.28 for ROP and 0.49 for controls). In contrast, uncoordinated development between the hyaloidal circulation and vitreous in the ROP animals was evidenced by a negative regression coefficient (−0.26), which was not the case for the control animals (regression coefficient, 0.34). We considered whether this evidence for uncoordinated growth reflects growth retardation of the ROP group compared with age-matched controls and not pathologic conditions per se. This does not seem to be the most likely explanation, because one would expect the ocular components to develop in a coordinated fashion throughout development. In addition, this uncoordinated development between hyaloidal circulation and vitreous is consistent with the greater irregularity observed for the hyaloidal shapes in day 12 ROP animals, the relatively greater asymmetry in the progression of the hyaloidal perfusion response, and the larger ratio of functional hyaloidal extent volume to vitreous volume (Figs. 4, 5). Taken together, these data suggest an abnormal ocular growth pattern in the ROP animals compared with controls. Although it is interesting to note that myopia, which is likely the result of uncoordinated growth of ocular components, is associated with children with mild ROP, care must be taken in applying the results of the study, using an experimental ROP model, to a clinical situation.

In summary, carbogen-enhanced MRI appears to be a powerful new and noninvasive approach for assessing the functionality of the hyaloidal circulation (that is, its ability to respond to carbogen challenge) and quantitatively comparing the functional hyaloidal extent to other ocular volumes in the same eye during development and disease. Evidence is presented here for the first time that supports our hypotheses that the function of the hyaloidal circulation is impaired and that the growth of ocular components is relatively uncoordinated in experimental ROP animals compared with age-matched controls.

References