Complicated colobomatous microphthalmia in the microphthalmic (mi/mi) mouse

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Summary

A study of the development of the eye in the cinnamon mouse, homozygous for the gene for microphthalmia (mi), has shown that the microphthalmia is due to failure of secondary vitreous formation associated with a coloboma. The retina is dystrophic but there is a residual population of large ganglion cells and the optic nerve also contains ganglion cells. All these ganglion cells have cytoplasm similar to the retinal ganglion cells in the normal controls. It is postulated that they communicate with axons in the optic nerve. In addition, the outer epithelial layer of the eye cup, which normally becomes pigmented, forms retinal tissue in the homozygous mouse and this is also true of the dorsal part of the eyestalk near the eye.

Key words: microphthalmia, retina, dystrophy, mouse, coloboma, mutant.

Introduction

The resorption of the mesenchyme between the optic vesicle and the ectoderm is important in the normal development of the eye as failure of resorption of mesenchyme prevents ectodermal–vesicle apposition (Silver & Hughes, 1973, 1974). When sufficient mesenchyme persists, it proliferates and the optic rudiment is subsequently resorbed. Should the lens form and reach a critical size in the mouse (Silver & Hughes, 1974) and rat (Kinney, Klintworth, Lesiewicz, Goldsmith & Wilkening, 1982), it is retained within the cup and the eye becomes microphthalmic.

The normal spherical expansion of the eye depends on the increase of intraocular volume due to vitreous production (Coulombre, 1956). Previous studies have suggested that no vitreous is formed in the homozygous (mi/mi) mouse (Konyukhov & Osipov, 1968), while Muller (1951) postulated that it formed, but leaked into the orbit through a coloboma, a common association of microphthalmia.

Microphthalmia can occur in association with a number of ocular defects: coloboma, optic nerve hypoplasia and retinal degeneration (Wyse & Hollenberg, 1977). When all these defects occurred in the rat, these authors called the syndrome complicated colobomatous microphthalmia. Similar abnormalities have been described in the eye of the microphthalmic (mi/mi) mice used in our study (see Konyukhov & Osipov, 1968). The normally situated retina is thin, lacking rods and is abnormal in the central portion (Konyukhov & Osipov, 1968). In addition in the dorsal eye cup, which normally develops into pigment epithelium (PE) and from which pigment is absent, there is an area that appears to be a mirror image of the subjacent retina (Konyukhov & Osipov, 1968).

There are no studies similar to those in the rat (Wyse & Hollenberg, 1977) on development of the eye of a mouse with complicated colobomatous microphthalmia. Herein we report the development of the eye of the mouse homozygous for the gene for microphthalmia (mi); this was studied by measuring the volume of the lens as a means of determining the extent of contact between the optic vesicle and surface ectoderm. The production of the secondary vitreous was studied by measuring the volume of the globe; the extraocular contents were studied to see if vitreous escaped through the coloboma. The retina was also examined to see if it was possible to identify ganglion cells which might communicate with the optic nerve. The nerve in turn was studied by measuring its myelination, cross-sectional area and the number of myelinated fibres.
Materials and methods

The gene for microphthalmia (mi) is pleiotrophic, producing manifold effects on fur, eyes and skeleton (Gruneberg, 1952) and is present at the microphthalmia locus on chromosome 6 (Silvers, 1979); it is semidominant. In heterozygous (mi/+ mouse there is spotting of the fur as melanocytes fail to migrate from the neural crest (Mintz, 1969). There is focal pigmentation of the retinal pigment epithelium (PE) and of the choroid in the heterozygous (mi/+ mouse (Deol, 1971). The homozygous (mi/mi) mouse is white with absence of pigment due to the total absence of melanocytes (Mintz, 1969). Homozygous (mi/mi) mice lack teeth and, postweaning, were fed on ground rat cubes and wheat germ.

Embryos were obtained from ‘time pregnancies’ where the observation of a vaginal plug was taken as day E1, of matings of both mice cinnamon and mice heterozygous (mi/+ for the microphthalmia gene. The mothers were killed by cervical dislocation and embryos were obtained at each stage at 8h intervals from day E10 to day E18; embryos of cinnamon matings were also obtained on day E19. After removal all embryos were immediately immersed in Lillies' fixative (Barash & Shepard, 1976) for a minimum of 24 h. This fixative facilitates preservation with good cellular detail and of cells in mitosis. The embryos to be studied histologically were embedded in paraffin and 8 μm serial sections cut and stained with haematoxylin and eosin. The day E10 and E11 embryos were cut coronally to obtain the best-aligned sections of the optic vesicle. Those from day E11-5 to day E19 were cut parasagittally, coronally and in line with the optic nerve which is along a plane perpendicular to the eyelid.

One mouse of each of the three phenotypes (cinnamon, heterozygous (mi/+), homozygous (mi/mi)) was killed postnatally (P) at ages 2, 7, 10, 12, 15, 35 days and at 3 months, by intracardiac perfusion of Lillies' fixative. Two adults of each phenotype were also studied. The eye was dissected out and fixed and 8 μm sagittal serial sections were cut and stained as previously described.

The volume of the lens and globe was measured in both embryos and postnatal mice by projecting the image of the sagittal section of the globe with maximum diameter, through a side arm attached to a microscope onto a graphics tablet using the x10 objective.

The intracranial portion of the optic nerve from six adult mice was removed, fixed in formalin for 24h and embedded in paraffin; 8μm thick sections were stained by the Luxol fast blue method (Kluver & Barrera, 1953) and the densitometric facility on the Quantimet 720 used to determine the amount of Luxol fast blue/myelin complex present (Scholtz, 1977). The cross-sectional area was determined as described for the lens and globe.

A sample of the intracranial portion of the optic nerve of one of each of the three groups of mice was obtained from animals killed by glutaraldehyde perfusion while under ether anaesthesia. Tissues were plastic-embedded and examined with a Hitachi 500 transmission electron microscope, a montage prepared, and the number of myelinated nerve fibres determined.

Results

The optic vesicle formed during the latter part of day E10, in association with apoptosis and resorption of the intervening mesenchyme as it approached and contacted the overlying ectoderm (arrows, Fig. 1). The surface ectoderm thickened in all embryos and invaginated to form the lens cup, which was directly superimposed on the optic cup (Fig. 1).

Early on day E12 it was possible to identify the homozygous (mi/mi) mice, as the other two groups (cinnamon and heterozygous (mi/+)) had early pigmentation of their PE (PE, Fig. 2); this zone of pigmentation extended into the dorsal portion of the distal optic stalk on day E13 (arrow, Fig. 2).

In the homozygous (mi/mi) mice the original outer layer of the eye cup had thickened and appeared as a mirror image of the subjacent retina (PE, Figs 3, 4). This thickening extended into the distodorsal optic stalk (arrows, Fig. 3) in a manner similar to the normal transient pigmentation of this area in the cinnamon and heterozygous (mi/+).

In the cinnamon and heterozygous (mi/+ mice the optic fissure began to close early on day E12. This did not occur in the homozygous (mi/mi) mice, where a coloboma formed (C, Fig. 4).

The ventral portion of the eye started to expand late on day E12 and early on day E13 only in cinnamon and heterozygous (mi/+ mice; this was associated with development of secondary vitreous (V, Fig. 5). During this stage the lips of the coloboma overlapped in the homozygous (mi/mi) mice (C, Figs 4, 6). No vitreous was apparent outside the globe.

On the vitreal surface of the retina in all embryos, extracellular spaces appeared late on day E11 (arrow, Fig. 4) and later in the day these were apparent in the optic stalk. These were not seen in the dorsal outer layer of the eye cup which resembled retina in the homozygous (mi/mi) mouse (Fig. 7). As the neurites left the eye they passed around the hyaloid artery and then entered the optic stalk but not the pigmented part of the distodorsal optic stalk in the cinnamon or heterozygous (mi/+ mice (Fig. 2) or the dorsal eye stalk which resembled retina in the homozygous (mi/mi) mice (Fig. 6). In the latter group neurites in the coloboma passed ventrally to form bundles in relation to the distal optic stalk (N, Fig. 6). Bundles of neurites formed in the dorsal eye cup which resembled retina in the homozygous (mi/mi) mice (arrows, Fig. 7). By late day E13 the optic nerve was well formed in all animals.

The retina began to differentiate in all mice on day E14. Initially, ganglion cells with large vesicular nuclei were apparent as a layer on the vitreal surface of the retina (gv, Fig. 8) and similarly in the dorsal eye cup which resembled retina in the homozygous
Fig. 1. Day E10 + 16 h. Coronal section of the optic vesicle to show thickening of the ectoderm and underlying vesicle (arrows). H&E x96.

Fig. 2. Day E13 + 0h. Sagittal section of dorsal optic vesicle and stalk. There are melanin granules present in the dorsal pigment epithelium (PE) and dorsal optic stalk (arrow). H&E x150.

Fig. 3. Day E12 + 16 h. Sagittal section of the eye from a homozygous (mi/mi) mouse embryo where the dorsal eye cup (PE) is thickened to resemble the subjacent retina and is starting to fold. This is also retinal differentiation in the dorsal optic stalk (os). The globe has not expanded, primary vitreous (V) is present but there is no formation of secondary vitreous. H&E x300.

Fig. 4. Day E12 + 16 h. Microphotograph of a coronal section of the eye of a homozygous (mi/mi) mouse. The dorsal eye cup is thickened to resemble the subjacent retina (PE). There are extracellular spaces on the vitreal surface of the retina (arrow). The optic tissie has not fused (C). H&E x150.

(mi/mi) mouse under the choroid (arrows and gs, Fig. 8). The PE in the cinnamon and heterozygous (mi/+ ) embryos was flattened, and ventrally was cuboidal in heterozygous (mi/mi) embryos (PE, Figs 8, 9). In the retina of cinnamon and heterozygous (mi/+ ) embryos there was thinning of the nuclear layers as the eye expanded but this did not occur in homozygous (mi/mi) embryos where the folding of the retina became more complicated (Fig. 10) and disorganized (R, Fig. 8). Debris accumulated within the retina (d, Fig. 10) and was abundant between the retina and the outer epithelial layer of the eye cup that developed into retina (d, Fig. 8). An inner plexiform layer developed (ipl, Fig. 8) both in the normally placed retina and dorsal eye cup that developed into retina. Pyknotic ganglion cell nuclei were seen only transiently on day P7 in the cinnamon and heterozygous (mi/+ ) mice but were present at all stages after day P7 in the homozygous (mi/mi) mice. Cytoplasm was observed in some of the ganglion cells of the centrally placed retina in the homozygous (mi/mi) mice. Cytoplasm was observed in some of the ganglion cells of the centrally placed retina in the homozygous (mi/mi) mice. Cytoplasm was observed in some of the ganglion cells of the centrally placed retina in the homozygous (mi/mi) mice. Cytoplasm was observed in some of the ganglion cells of the centrally placed retina in the homozygous (mi/mi) mice. Cytoplasm was observed in some of the ganglion cells of the centrally placed retina in the homozygous (mi/mi) mice.
Fig. 5. Day E13 + 0h. Sagittal section of eye. The pigment epithelium (PE) has thinned and the globe is expanding with the formation of secondary vitreous (V). Neurites (arrow) are present in the posterior pole of the eye and optic stalk. H&E ×120.

Fig. 6. Day E16 + 8h. Sagittal section of eye from homozygous (mi/mi) mouse. The retina is thick and there is little vitreous evident. The optic nerve is well formed (on). The dorsal eye cup (PE) is thick. The two lips of the coloboma have overlapped (C) and bundles of neurites (N) can be seen between these two lips. H&E ×96.

Fig. 7. Birth. Dorsal eye cup showing retinal differentiation in the eye of a homozygous (mi/mi) mouse. Bundles of neurites are present deep to the scleral surface (arrows). H&E ×360.

Fig. 8. Day 10 (P). Peripheral normally placed retina in microphthalmic (mi/mi) eye and dorsal eye cup which has differentiated into retina (arrows). Cuboidal pigment epithelium is also evident (arrow PE). A ganglion cell layer (g, gv) and inner plexiform layer (ipl) is evident in both the dorsal eye cup and retina. There is also disorganization of the normally placed retina (R). Debris (d) is present between the dorsal eye cup and retina.

Blood vessels were present in the choroid and the hyaloid artery was well formed in all groups of animals.

Once the anterior lens pore has closed at day P12, fibres formed within the lens. The first apparent difference between the lenses was the irregularity of the lens fibres in the homozygous (mi/mi) mouse. These initially degenerated by vacuolation of the anterior lens fibres and this change progressed to involve the entire lens by P35 day; in adult life this was calcified (L, Fig. 11) and was much smaller than the other two groups on volumetric measurement (Table 1). On day P10, ganglion cells, many with visible cytoplasm, were evident in the optic nerve (arrow, Fig. 12) and in the coloboma of the homozygous (mi/mi) mouse. After birth the vitreous of the homozygous (mi/mi) mouse was densely eosinophilic (V, Fig. 11) and at no stage as loosely textured as in the other groups; at no stage was vitreous seen outside the globe in the homozygous (mi/mi) mice.

There was no significant difference between the volumes of the lens or the globe in the homozygous (mi/mi), cinnamon or heterozygous (mi/+ ) embryos (Fig. 13). The major expansion occurred between birth and 10 days (Table 2). In adult life the difference between the homozygous (mi/mi) mice and the cinnamon and heterozygous (mi/+ ) mice was significant.
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Fig. 9. Day 2 (P). Sagittal section of homozygous (mi/mi) mouse eye. There is folding of the retina which encloses hyaline primary vitreous (V). The inner plexiform layer is apparent both in the retina and dorsal eye cup which has differentiated into retina (arrows). The ventral eye cup is cuboidal (PE) resembling normal pigment epithelium. H&E ×60.

Fig. 10. Day 12 (P). Central retina of microphthalmic (mi/mi) eye. Vesicular ganglion cell nuclei, some of which have cytoplasm, are apparent (arrow). There is focal accumulation of debris (d) in the disorganized retina. H&E ×480.

Fig. 11. Low-power photomicrograph of eye from microphthalmic (mi/mi) mouse. The lens (L) is calcified, the retina (R) dystrophic, the optic nerve (on) well developed and there is a bundle of neurites (N) present on the ventral aspect. There is a small amount of vitreous (V) evident. H&E ×30.

Fig. 12. Section of optic nerve from 10-day-old (P) microphthalmic (mi/mi) eye. Ganglion cells are present with cytoplasm (arrow). H&E ×300.

Table 1. Study of adult animals

<table>
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<tr>
<th></th>
<th>Cinnamon</th>
<th>(mi/+)</th>
<th>(mi/mi)</th>
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<tbody>
<tr>
<td>Volume of lens (×10⁹ μm³; N = 6)</td>
<td>1512.0 ± 422.9</td>
<td>1524.6 ± 299.9</td>
<td>820.0 ± 425.96</td>
</tr>
<tr>
<td>Volume of globe (×10⁶ μm³; N = 6)</td>
<td>4958.8 ± 974.6</td>
<td>5114.8 ± 257.33</td>
<td>1104.3 ± 615.68</td>
</tr>
<tr>
<td>Cross-sectional area of optic nerve, A (μm²; N = 6)</td>
<td>287.97 ± 31.16</td>
<td>204.55 ± 6.03</td>
<td>163.6 ± 22.00</td>
</tr>
<tr>
<td>Myelin in optic nerve, M (arbitrary units; N = 6)</td>
<td>125.78 ± 45.18</td>
<td>100.72 ± 71.83</td>
<td>48.37 ± 3.90</td>
</tr>
<tr>
<td>M/A</td>
<td>0.47</td>
<td>0.48</td>
<td>0.29</td>
</tr>
</tbody>
</table>

The results of the study of the eye and optic nerve (±S.D.) in the adult cinnamon, heterozygous (mi+/+) and homozygous (mi/mi) mouse.

(Table 1) reflecting the absence of secondary vitreous in the former.

There was no difference between the amounts of myelin–dye complex per unit area in the optic nerve in the cinnamon and heterozygous (mi+/+) mice (Table 1). The homozygous (mi/mi) mouse had
considerably reduced amounts of myelin–dye complex (Table 1). There was also a small area of necrosis noted in the centre of the nerve of the homozygous \((mi/mi)\) mouse in sections stained with Luxol fast blue. Electron microscopy showed this to consist of necrotic axons and comprised 21.1% of the cross-sectional area (Fig. 14). There was no evidence of macrophage activity in this area.

The cross-sectional area of the optic nerve in the heterozygous \((mi/+\) mouse was slightly smaller than the cinnamon mouse but the amount of myelin–dye complex per unit volume was the same (Table 2). Both these variables were greatly reduced in the homozygous \((mi/mi)\) mouse (Table 1). The number of myelinated fibres collected in the cinnamon mouse was 23,456; in the heterozygous \((mi/+\) mouse it was 22,603 and in the homozygous \((mi/mi)\) mouse 16,142.

Discussion

The lens of the microphthalmic \((mi/mi)\) mouse formed normally and was normal in size indicating that there was total resorption of the mesenchyme between the optic vesicle and the surface ectoderm as shown in other studies of microphthalmic animals (Silver & Robb, 1979; Wyse & Hollenberg, 1977). The coloboma formed in the ventral optic vesicle at the site of closure of the optic fissure (see also Silver & Hughes, 1973, 1974 for the mouse and Kinney et al., 1982 for the rat).

In the homozygous \((mi/mi)\) mouse the dorsal epithelial layer of the eye cup was replaced by retina. This abnormality extended into the dorsal distal optic stalk in a manner similar to that seen for the transient pigmentation in the control mice in both our work and in other studies (Silver & Robb, 1979). This area of retina lacked the extracellular spaces that were present in the normally situated retina of all mice, a change that resulted in the accumulation of bundles of neurites both subjacent to and within the retina. This supports the suggestion of other workers who emphasize the role of these extracellular spaces in the guidance of neurites (Silver & Robb, 1979; Silver & Sapiro, 1981).

The present study has shown that the cause of the microphthalmia is failure of formation of secondary vitreous. The primary vitreous developed in the homozygous \((mi/mi)\) mouse at the same time as the cinnamon and heterozygous \((mi/+\) mice and persisted into adult life, which conflicts with the observation of Konyukhov & Osipov (1968) who reported that vitreous did not form. Furthermore vitreous was not observed outside the globe, a possibility advanced as the cause of the microphthalmia (Muller, 1951). In addition this author suggested that retina was extruded through the coloboma. Our study indicates that the ‘extruded retina’ was dorsal eye cup that developed into retina.

The homozygous \((mi/mi)\) mouse had optic nerve hypoplasia in association with a reduced number of fibres in the optic nerve. This resulted from deflection of the developing neurites towards the sclera and fewer neurites reaching the optic nerve similar to the study of Goldberg & Frank (1979). Microphthalmia also caused disruption of the topographic organization by failure of rotation of the eye. Thus the hyaloid artery was placed ventrally and not in a direct line from the eye to the brain, and there was failure of neurites to enter the optic stalk. A similar accumulation of neurites has been described when the glial sling is cut prior to the formation of the nerve fibres in the corpus callosum and in mice with aplasia of the corpus callosum (Silver, Lorenz, Wahlsten & Coughlin, 1982).

Our work has shown that the homozygous \((mi/mi)\) mouse has a recessively inherited retinal dystrophy similar to that described in the BW rat where there is associated microphthalmia, retinal dystrophy and cataract formation (Wyse & Hollenberg, 1977). The histological features of retinal dysplasia are non-specific and occur when retinal growth is disturbed for whatever cause (Lahav, Albert & Wyand, 1973). The accumulation of debris within the retina and between the retina and the dorsal layer of the eye cup that developed into retina was similar to the RCS rat.
Table 2. **Volume of lens and globe**

<table>
<thead>
<tr>
<th>Postnatal day</th>
<th>Normal Lens</th>
<th>Normal Globe</th>
<th>Abnormal Lens</th>
<th>Abnormal Globe</th>
</tr>
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<tbody>
<tr>
<td>2</td>
<td>541±88</td>
<td>2967±43</td>
<td>245±107±44</td>
<td>2203±41±246±97</td>
</tr>
<tr>
<td>7</td>
<td>1132±54</td>
<td>5012±49</td>
<td>1000±43</td>
<td>5317±81</td>
</tr>
<tr>
<td>10</td>
<td>1361±27</td>
<td>6358±96</td>
<td>1337±47</td>
<td>6538±96</td>
</tr>
<tr>
<td>12</td>
<td>1292±38</td>
<td>5696±57</td>
<td>1037±10</td>
<td>5696±57</td>
</tr>
<tr>
<td>15</td>
<td>1216±69</td>
<td>5110±40</td>
<td>1185±12</td>
<td>5110±40</td>
</tr>
<tr>
<td>35</td>
<td>2391±67</td>
<td>5075±56</td>
<td>1818±68</td>
<td>4626±23</td>
</tr>
</tbody>
</table>

The volume of the lens and globe ($\times 10^3 \mu m^3$) from the cinnamon, heterozygous (mi/+), and homozygous (mi/mi) postnatal mice on the days indicated. For the heterozygous matings the postnatal mice on day two include 6 cinnamon and heterozygous (mi/+), mice ($\pm S.D., S.E.$).

![Fig. 14. Electron microphotograph of the optic nerve of a homozygous (mi/mi) mouse showing central necrosis. EM x11500.](image)

where the pigment epithelium lacked the ability to phagocytose the outer segments of the rods (Bok & Hall, 1969, 1971; Mullen & LaVail, 1975). This lamellar debris may have acted as a barrier between the choroid and the photoreceptor cells resulting in their poor nutrition as postulated by Herron, Riegel, Myers & Rubin (1969). Slowing of retinal degeneration has been reported in starved C3H mice (Lucas & Newhouse, 1957) and this may have occurred in this study as the microphthalmic mice had no teeth, a reduced ability to eat and so were probably malnourished.

The ocular blood vessels appeared normal and so it is not possible to attribute the pathology to vascular abnormalities. Ocular vascular abnormalities have been related to microphthalmia (Browman, 1961) but in that instance there was total absence of the hyaloid artery.
The ganglion cells in the peripheral retina of the homozygous (mi/mi) mouse lacked cytoplasm as did those in the dorsal eye cup that resembled retina. The population of ganglion cells identified in the centrally placed retina and optic nerve with cytoplasm in these mice was an unexpected finding. It is suggested that these ganglion cells send axons into the optic nerve while those in the dorsal eye cup and peripheral retina do not. The reduced numbers of axons and diminished myelination of the optic nerve in the homozygous (mi/mi) mouse correlates with the reduced number of retinal ganglion cells with cytoplasm. Reduced myelination of the optic nerve suggests that they have little function and are similar to those of adult mice reared in darkness (Gyllensten & Malmfors, 1963).

References


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