Unilateral Atrophy of the Optic Nerve Associated with Retrograde and Anterograde Degenerations in the Visual Pathways in Slc: Wistar Rats

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(Received 21 April 1993/Accepted 25 June 1993)

ABSTRACT. Unilateral degenerative atrophy of the optic nerve (ON) occurred in 6 of 80 male and 4 of 80 female Slc: Wistar rats. Two of these cases completely lost the intracranial portion of the unilateral ON and the remainder had the small ON. The optic disc and ON were located histologically in the posterior pole of the eyeball of 2 rats with no intracranial ON. ON lesions in all cases were characterized by a reduced number of axons with a small number of myelinated axons and marked astrogliosis. There were also swelling, fragmentation and spheroid formation of axons, as well as thickening of the connective tissue sheaths and vessel walls in the ON. One side of the optic chiasma and optic tract contralateral to the affected ON reduced in volume, became degenerated and were accompanied by gliosis. Focal or diffuse degeneration of the retina was observed in the eyeballs with affected ON. Retinal ganglion cells decreased in the number showing chromatolysis. These retinæ became thin and developed degeneration of both inner and outer portions with sclerotic changes in the retinal vessels. The ophthalmic and ciliary arteries in the eyeballs with affected ON often developed proliferative or occlusive endoarteritis, suggesting that retinal lesions may have resulted not only from axonal degeneration in the ON but also from ischemia. Histologic lesions suggestive of transneuronal degeneration were found in the contralateral lateral geniculate body and rostral colliculus. Based on the data presented, it was presumed that a primary lesion may have been induced in the ON by a circulatory disturbance and followed by retrograde and anterograde degenerations in the visual pathways.—**KEY WORDS:** anterograde degeneration, optic nerve pathway, rat, retrograde degeneration, unilateral atrophy.

J. Vet. Med. Sci. 55(6): 905–912, 1993

Atrophy of the optic nerve (ON) in animals has been described to occur as the results of inflammatory or non-inflammatory changes in the ON as well as killing of the retinal ganglion cells by various agents such as toxin and virus [18]. Spontaneously occurring atrophy of the ON is rare in rats, and little is known about its pathogenesis. Lee *et al.* [11] reported spontaneous unilateral degeneration of the retina and ON in F-344 rats. They suggested that the condition may be accelerated by the feeding of certain semi-purified synthetic diets, but the precise pathogenesis of the lesions remained to be determined. A decrease in the number of retinal ganglion cells and thinning of the ON with astrogliosis have been induced in albino Wistar rats by crushing the ON [9]. This paper describes unilateral degenerative atrophy of the ON in Slc: Wistar rats with emphasis on morphologic changes in the visual pathways.

MATERIALS AND METHODS

Eighty male and 80 female Slc: Wistar specific-pathogen-free rats were purchased from Shizuoka Laboratory Animal Center (Shizuoka) at the age of 4 weeks. They were maintained up to 57 weeks of age in a barrier-sustained animal room, controlled at $23 \pm 2^\circ\text{C}$ and $50 \pm 20\%$ relative humidity and illuminated by white fluorescent ceiling lights with a 12 hr light-dark cycle. The rats were housed individually to wire mesh cages ($21 \times 35 \times 20$ cm) which were hanged to the rack containing five rows of four cages. The light intensity within each cage in the racks ranged from 38 to 250 lux depending on a position of the cages. The rats had free access to a

standard laboratory diet for rats (CRF-1, Charles River Japan, Inc., Kanagawa) and tap water. Ophthalmological examinations were performed with a direct ophthalmoscope (Welch Allyn, Inc., NY, U.S.A.) and a fundus camera (RC-2 model-621, Kowa, Tokyo) at 5, 31, and 57 weeks of age. At the age of 57 weeks, all rats were bled under the deep anesthesia and subjected to a complete necropsy. The eyeballs were fixed in Bouin's solution. The ON and brains were fixed in 10% neutral buffered formalin. The anatomic regions of the optic chiasma, optic tract, lateral geniculate body (LGB), and rostral colliculus (RC) referred to the atlas of the rat brain [15, 16]. The specimens were embedded in paraffin wax, sectioned and stained with hematoxylin and eosin (H & E). Selected sections were stained with periodic acid-Schiff (PAS), azan-Mallory, and phosphotungstic acid hematoxylin (PTAH), and by the Bodian and Klüber-Barrera (KB) methods. Paraffin-embedded sections were stained immunohistochemically by the peroxidase-antiperoxidase (PAP) technique with a commercial kit (DAKO PAP Kit System, DAKO Corporation, CA, U.S.A.). Prediluted rabbit anti-glial fibrillary acidic protein (GFAP) and neuron-specific enolase (NSE) antibodies (DAKO Corporation) were used as primary antibodies. The total ganglion cells in the ganglion cell layer of the retina were counted in a vertical section cut at an optic disc level of the eyeball. The ganglion cells in the histologically normal retina from 10 healthy, age-matched Slc: Wistar rats were also counted in the identical manner and served as controls. The statistical significance was assessed by Student's *t*-test for difference in the number of ganglion cells between the affected retina and the normal contra-

Fig. 1. Fundus photograph. Case no. 7, the right fundus showing diffuse hyperreflectance of the retina and narrowness of the central retinal arteries and veins.

lateral retina and the retina of normal controls.

RESULTS

Ophthalmology: None of the rats examined manifested any clinical signs during the observation period and abnormal fundusoscopic changes at the age of 5 weeks. When examined at the age of 31 weeks, 3 males (case nos. 2, 4, and 5) and one female (case no. 8) had unilateral retinal atrophy. At the age of 57 weeks, 3 other males (case nos. 1, 3, and 6) and 3 other females (case nos. 7, 9, and 10) developed unilateral retinal atrophy. The total incidence of retinal atrophy was 7.5% (6/80) in males and 5.0% (4/80) in females (Table 1). Ophthalmoscopically, the atrophic retina was recognized as a focal or diffuse hyperreflectance of the fundus, narrowness of the retinal arteries and veins, and mild swelling of the optic disc (Fig.

Fig. 2. Ventral surface of the brain. a. Case no. 3, the right optic nerve reduces its size and is semitransparent compared to the normal-appearing left optic nerve, and the left optic tract is barely visible. b. Case no. 1, the right optic nerve is completely missing and the left optic tract reduces in volume. The vestige of the right optic nerve is seen on the surface of the optic chiasma (arrowhead). $\times 8$.

1). The degree of retinal atrophy appeared to progress with increasing age of the rats. No abnormalities were detected ophthalmoscopically in other parts of the eye and no relationship existed between development of unilateral retinal atrophy and a position of the cages in the racks, i.e. the intensity of light.

Gross pathology: All cases with unilateral fundusoscopic lesions exhibited atrophy of the ipsilateral ON and of the contralateral optic tract (Fig. 2). The intracranial portion of the unilateral ON was completely missing in 2 rats (case

Table 1. Gross findings of the optic nerve and fundusoscopic lesions in the eye with optic nerve atrophy in Slc: Wistar rats

Case no.	Sex	State of the optic nerve	Side	Fundal lesions in the eye with optic nerve atrophy	Weeks of age when fundal lesions detected
1	Male	Absent	Right	Diffuse atrophy	57
2	Male	Atrophic	Left	Diffuse atrophy	31
3	Male	Atrophic	Right	Diffuse atrophy	57
4	Male	Atrophic	Left	Focal atrophy	31
5	Male	Atrophic	Right	Diffuse atrophy	31
6	Male	Atrophic	Right	Diffuse atrophy	57
7	Female	Absent	Right	Diffuse atrophy	57
8	Female	Atrophic	Right	Diffuse atrophy	31
9	Female	Atrophic	Right	Focal atrophy	57
10	Female	Atrophic	Left	Diffuse atrophy	57

Fig. 3. The optic nerve adjacent to the posterior pole of the eyeball of case no. 7. $\times 80$. a. Note atrophy and marked astrogliosis, H & E; b. Note markedly decreased number of myelinated axons, KB; c. Note faint positive reaction for NSE, PAP method for NSE; and d. Note marked glial proliferation infiltrating into the optic disc, PAP method for GFAP.

nos. 1 and 7), leaving the vestige of the ON on the surface of the optic chiasma (Fig. 2b, arrowhead). Those eyeballs which had ON atrophy were similar in size to the normal contralateral eyeballs.

Histopathology: In the 2 eyeballs that lost intracranial part of the ON (case nos. 1 and 7), the optic disc and the ON were found adjacent to the posterior pole of the eyeball. ON lesions in the posterior poles of the eyeballs of these 2 cases and in the other 8 cases with the small ON were characterized by a markedly reduced number of axons, myelin breakdown and the increased number of astrocytes. Remarkably increased glial fibers were demonstrated by the GFAP immunostaining. Immunoreactivity for NSE in axons of the atrophic ON was evidently weaker than that in the normal contralateral ON (Fig. 3). Axons in the optic disc also decreased in the number with infiltration of many astrocytes. Degenerated axons were fragmented or swollen and appeared to be tortuous lines. Irregularly shaped spheroids containing several PAS-positive granules were occasionally observed (Fig. 4). Similar changes were also present in the intracranial portion of the atrophic ON. Some macrophages containing a few PAS-positive granules in their cytoplasm were seen in the atrophic ON. The leptomeninges, connective tissue septa and vascular walls in the atrophic ON became thickened due to proliferation of collagenic fibers (Fig. 5).

Degenerative changes of the retina were detected in all the eyeballs with the small ON, whereas no noticeable changes were seen in the cornea, iris, ciliary body, and lens in these eyeballs as well as in any structures of the normal contralateral eyeballs. Two eyeballs (case nos. 4 and 9) had focal degeneration of the retina while others showed diffuse degeneration of the retina (Fig. 6). The extent of retinal changes well coincided with the fundusco-

Fig. 4. The intracranial portion of the atrophic optic nerve. Case no. 3. $\times 300$. a. Note marked loss, tortuosity, fragmentation, and spheroid formation of axons, and astrogliosis. Bodian; b. PAS-positive granules are seen in axonal spheroids (arrows) and in the cytoplasm of macrophages (arrowheads). PAS.

pic findings. The retinal lesions consisted of thinning, fragmentation, and destruction of the whole retinal layers. The layer of rods and cones was remarkably thin or lost, and nuclei of the outer and inner nuclear layers greatly

Fig. 5. The intracranial portion of the optic nerve, case no. 10. a. Note marked proliferation of collagenic fibers in the pial sheath, septa, and vascular walls in the atrophic optic nerve; b. No fibrous thickening is seen in the normal contralateral optic nerve. Azan-Mallory, $\times 100$.

Fig. 6. Posterior part of the eye. a. Case no. 1, the right retina is diffusely atrophic, and most of the outer layers have been lost. $\times 30$. b. Case no. 4, the left retina shows focal atrophy with some parts of the outer layers being lost. $\times 80$. H & E.

Fig. 7. Retina, case no. 7. a. Atrophic retina showing marked loss, chromatolysis and other degenerative changes of the ganglion cells. b. No such changes are seen in the normal contralateral retina. KB, $\times 400$.

Table 2. The numbers of ganglion cells in the retinal ganglion cell layers of the eyes with unilateral optic nerve atrophy, the normal contralateral eyes and the healthy control eyes of Slc: Wistar rats aged 57 weeks

Condition	Number of eyes examined	Number of ganglion cells	Range of the numbers of ganglion cells
Eye with optic nerve atrophy	10	235 ± 68^b	132-345
Normal contralateral eye	10	$551 \pm 59^{***}$	462-628
Healthy control eye ^{a)}	10	$535 \pm 50^{***}$	484-634

a) Eyes from healthy, age-matched Slc: Wistar rats.

b) Values represent mean $\pm$ standard deviation.

***Significantly ($p < 0.001$) different from the mean of the eyes with optic nerve atrophy.

decreased in the number. The number of retinal ganglion cells in the eyes with affected ON was significantly smaller than that in the normal contralateral eyes and the healthy control eyes (Table 2). Most of ganglion cells surviving in the retina exhibited chromatolysis or other degenerative changes (Fig. 7). The nerve fiber layer markedly reduced its thickness and showed a faint immunoreactivity for NSE. In the degenerated retina, the walls of the superficial retinal vessels and capillaries became thickened. The deposition of hyaline material and proliferation of collagenic fibers in the thickened walls were demonstrated by PAS and azan-Mallory stainings.

Table 3. Relationship between degrees of arterial lesions and lesion scores in the retina and optic nerve

Case no.	Degree of occlusive lesions in ^{a)}		Lesion score ^{b)c)} in the inner portion of the retina	Lesion score ^{b)d)} in the outer portion of the retina	Lesion ^{b)} score in the optic nerve
	Ophthalmic artery	Ciliary artery			
1	+++	—	+++	+++	+++
2	+	—	++	++	++
3	+	—	++	++	++
4	—	—	+	+	++
5	+++	+	++	++	++
6	NA	NA	+++	+++	++
7	+++	+	+++	+++	+++
8	+	+	++	++	++
9	NA	—	+	+	++
10	NA	—	++	++	+

a) Lesions are graded on a scale of +, less than 30%; ++, 30 to 50%; and +++, more than 50% occlusion of the vessel lumen.

b) Degenerative changes in the retina and optic nerve are graded on a scale of + (slight), ++ (moderate), and +++ (severe).

c) Inner portion of the retina includes the inner limiting membrane and the layers of nerve fibers, ganglion cells, inner plexiform, inner nuclei, and outer plexiform.

d) Outer portion of the retina includes the outer nuclear layer, layer of rods and cones, and pigment epithelium.

NA: not available because of missing of the arteries.

Fig. 8. The ophthalmic artery. Case no. 7, proliferation of intima and medial smooth muscles, and fragmentation of the intimal elastic lamina have occluded the vascular lumen. PTAH, $\times 560$.

The ophthalmic and ciliary arteries in the eyeballs with affected ON had proliferative or occlusive endoarteritis. Out of the 10 cases examined, 6 (case nos. 1, 2, 3, 5, 7, and 8) revealed intimal proliferation of the ophthalmic artery, one (case no. 4) had the apparently normal artery, and in the remainder (case nos. 6, 9, and 10) the ophthalmic artery could not be located. The ciliary arteries of 3 (case nos. 5, 7, and 8) had intimal proliferation, those of 6 (case nos. 1 to 4, 9, and 10) appeared normal, and in the remainder (case no. 6), the artery could not be located. As shown in Table 3, degrees of occlusive lesions in the both arteries were generally correlative with lesion scores in the retina and optic nerve of the cases examined. In these arterial lesions, there were proliferation of collagenic fibers and the medial smooth muscles, deposition of

hyaline material, and fragmentation of the intimal elastic lamina (Fig. 8). The degrees of intimal proliferation varied from a slight protrusion into the vascular lumen to almost complete occlusion of the lumen.

One side of the optic chiasma and the optic tract contralateral to the affected ON markedly reduced in volume due to a decreased number of axons with a small number of myelinated axons and an increased number of astrocytes (Fig. 9). Fragmented and tortuous axons were also seen in these areas. Both contralateral LGB and RC became atrophic and contained a reduced number of nerve fibers. The ON layer in the affected RC was attenuated (Fig. 10). In these areas, atrophic and degenerative changes of the nerve cells and proliferation of astrocytes and microglia were noted (Fig. 11).

DISCUSSION

The ocular lesions described here were unilateral and involved the ON and the ipsilateral retina, as well as the contralateral optic tract and, LGB and RC of the brain. The 2 rats showing a complete loss of the unilateral ON reminded us of unilateral ON aplasia in rats [22, 23]. However, these cases clearly differed from the congenital anomaly in that the rats have lost only the intracranial portion of the unilateral ON, leaving its vestige on the surface of the optic chiasma and, the optic disc and the ON were recognizable adjacent to the posterior pole of the eyeball. Furthermore, ON lesions in all the 10 cases were characterized by axonal degeneration and reactive proliferation of glial cells and collagenic fibers. These findings definitely indicate that the ocular lesions reported here are due to degeneration and not due to aplasia or hypoplasia.

Fig. 9. Frontal section cut through the optic tract. Case no. 3. a. The left optic tract, contralateral to the affected optic nerve, reduces in volume and contains a small number of myelinated axons. b. The right optic tract appears normal. KB, $\times 30$.

Fig. 10. Frontal section cut through the rostral colliculus. Case no. 7. The left colliculus, contralateral to the affected optic nerve, reduces in volume with marked astrogliosis and the attenuated optic nerve layer (arrowheads). KB, $\times 50$.

Fig. 11. Higher magnification of part of the left colliculus shown in Fig. 10. Note atrophy and degeneration of the nerve cells and proliferation of both astrocytes and microglia. KB, $\times 500$.

Experimental studies using monkeys [17, 20], rats [9] and cats [5] have demonstrated that cutting and crushing of the ON in the orbit or eye enucleation resulted in descending and ascending ON degeneration, loss or degeneration of the retinal ganglion cells and fibers. From these observations, it seems conceivable that a primary lesion may have been induced in the ON of the present cases and followed by retrograde and anterograde degenerations in the visual pathways.

It is worth noting that proliferative or occlusive endoarteritis was often detected in the ophthalmic and ciliary arteries ipsilateral to the affected ON. Such vascular changes may also have played an important role in the development of the ocular lesions, and may explain why all the retinal layers have been involved in the present cases. This is because the ophthalmic artery supplies blood to the central retinal arteries and the ciliary arteries and subsequently the central retinal arteries nourish the inner layers of the retina and the ciliary arteries supply blood to the choriocapillaries that provide oxygen and nutrients to the pigment epithelium and the outer part of the retina [6]. Differing from the present cases, age-related and light-associated retinal lesions in rats have been reported to be confined mostly to the outer layers of the retina [1, 2, 10, 14, 19, 21, 25]. On the other hand, spontaneous unilateral ON aplasia [22, 23], unilateral degeneration of the retina and ON [11] and experimentally induced injury to the ON [9] mainly involved the inner portion of the retina, particularly the layers of ganglion cells and optic nerve fibers. Sclerotic changes of the retinal vessels and capillaries similar to those observed in the present cases have been described in Osborne-Mendel rats with age-related retinal degeneration [25], Chbb/THOM rats with age- and light-dependent retinal changes [26], and Long-Evans rats with phototoxic retinopathy [2]. Age-related polyarteritis characterized by degenerative, inflammatory and proliferative changes has been described to occur frequently in Sprague-Dawley rats [12]. In the present cases, however, no such arterial lesions were detected in any of the tissues examined, and the etiology of the occlusive lesions in the ophthalmic and ciliary arteries remains unsolved. Based on the presence of lesions in both ocular arteries, it is analogized that a primary lesion in the ON may have resulted from ischemia. It has been described that intracranial part of the ON of rats contains no large internal vessels and receives a blood supply via its meningeal covering [9]. Therefore, the pathogenesis of a probable primary lesion in the ON remains to be solved until the meningeal vessels can be examined in detail.

Lesions seen in one side of the optic chiasma, optic tract, LGB and RC contralateral to the affected ON were interpreted as anterograde degeneration. This is based on the fact that axons of the ON are the central projections of retinal ganglion cells and terminate in the contralateral LGB and RC after passing through the optic chiasma and optic tract [3, 24], and the uncrossed visual pathways to the optic center are small in the number in albino rats [13]. The presence of centrifugal fibers has been demonstrated

in the rat ON [7, 8]. Therefore, the affected optic tract may possibly be composed of axons from ipsilateral retinal ganglion cells and centrifugal axons from the brain in addition to degenerated axons from contralateral retinal ganglion cells. Histologic features observed in the affected LGB and RC of the present cases agreed well with those that have been described in transneuronal degeneration in the LGB resulting from lesions of the retina, ON or optic tract [4].

ACKNOWLEDGEMENTS. The authors thank Messrs. K. Ikura, M. Kaneko, and S. Ishikawa for their technical assistance, and Mrs. S. Sannai for her statistical analysis.

REFERENCES

1. Bellhorn, R. W. 1980. Lighting and the animal environment. *Lab. Anim. Sci.* 30: 440-450.
2. Bellhorn, R. W., Burns, M. S., and Benjamin, J. V. 1980. Retinal vessel abnormalities of phototoxic retinopathy in rats. *Invest. Ophthalmol. Visual Sci.* 19: 584-595.
3. Bunt, A. H., Lund, R. D., and Lund, J. S. 1974. Retrograde axonal transport of horseradish peroxidase by ganglion cells of the albino rat retina. *Brain Res.* 73: 215-228.
4. Duchen, L. W. 1984. General pathology of neurons and neuroglia. pp. 1-52. In: Greenfield's Neuropathology, 4th ed. (Adams, J. H., Corsellis, J. A. N., and Duchen, L. W. eds.), Edward Arnold Ltd., London.
5. Fillenz, M. and Gleeves, P. 1961. Degeneration of optic nerve in the cat. *J. Physiol.* 158: 18.
6. Hebel, R. and Stromberg, M. W. 1986. Anatomy and Embryology of the Laboratory Rat, BioMed Verlag, Worthsee.
7. Hanrubia, F. M. and Elliott, J. H. 1968. Efferent innervation of the retina. I. Morphologic study in human retina. *Arch. Ophthalmol.* 80: 98-103.
8. Itaya, S. K. 1980. Retinal efferents from the pretectal area in the rat. *Brain Res.* 201: 436-441.
9. Kiernan, J. A. 1985. Axonal and vascular changes following injury to the rat's optic nerve. *J. Anat.* 141: 139-154.
10. Lai, Y., Jacoby, R. O., and Jonas, A. M. 1978. Age-related and light-associated retinal changes in Fischer rats. *Invest. Ophthalmol. Visual Sci.* 17: 634-638.
11. Lee, E. W., Render, J. A., Garner, C. D., Brady, A. N., and Li, L. C. 1990. Unilateral degeneration of retina and optic nerve in Fischer-344 rats. *Vet. Pathol.* 27: 439-444.
12. Lewis, D. J. 1992. Nonneoplastic lesions in the cardiovascular system. pp. 301-309. In: Pathology of the Aging Rat, vol. 1 (Mohr, U., Dungworth, D. L., and Capen, C. C. eds.), ILSI Press, Washington, D. C.
13. Lund, R. D. 1965. Uncrossed visual pathways of hooded and albino rats. *Science* 149: 1506-1507.
14. O'Steen, W. K., Anderson, K. V., and Shear, C. R. 1974. Photoreceptor degeneration in albino rats: Dependency on age. *Invest. Ophthalmol.* 13: 334-339.
15. Palkovits, M. and Brownstein, M. J. 1988. Maps and Guide to Microsection of the Rat Brain. Elsevier, New York.
16. Paxinos, G. and Watson, C. 1986. The Rat Brain in Stereotaxic Coordinates, 2nd ed., Academic Press, Sydney.
17. Quinley, H. A., Davis, E. B., and Anderson, D. R. 1977. Descending optic nerve degeneration in primates. *Invest. Ophthalmol. Visual Sci.* 16: 841-849.

18. Saunders, L. Z. and Rubin, L. F. 1975. Ophthalmic Pathology of Animals. Kager S, Basel.
19. Schardein, J. L., Lucas, J. A., and Fitzgerald, J. E. 1975. Retinal dystrophy in Sprague-Dawley rats. *Lab. Anim. Sci.* 25: 323-326.
20. Schmidt, R. E., Tredici, T. J., and O'Connor, P. S. 1982. Histopathology of experimental optic tract hemianopia. *Ophthalmic Res.* 14: 354-360.
21. Semple-Rowland, S. L. and Dawson, W. W. 1987. Retinal cyclic light damage threshold for albino rats. *Lab. Anim. Sci.* 37: 289-298.
22. Shibuya, K., Tajima, M., and Yamate, J. 1992. Unilateral optic nerve aplasia in an F344 rat with special reference to pathomorphological changes of the optic nerve pathways. *J. Vet. Med. Sci.* 54: 571-574.
23. Shibuya, K., Tajima, M., Yamate, J., and Kudow, S. 1989. Unilateral optic nerve aplasia in two young Slc: Wistar rats. *Vet. Pathol.* 26: 518-820.
24. Slatter, D. 1990. Structure and function of the eye. pp. 1-31. In: Fundamentals of Veterinary Ophthalmology, 2nd ed., W. B. Saunders Company, Philadelphia.
25. Von Sallmann, L. and Grimes, P. 1972. Spontaneous retinal degeneration in mature Osborne-Mendel rats. *Arch. Ophthalmol.* 88: 404-411.
26. Weisse, I., Stotzer, H., and Steitz, R. 1974. Age- and light-dependent changes in the rat eye. *Virchows Arch. A Pathol. Anat. and Histol.* 362: 145-156.