GERONTOLOGY

Clinical and Morphological Characteristics of Chorioretinal Degeneration in Early Aging OXYS Rats

A. A. Zhdankina, A. Zh. Fursova*, S. V. Logvinov, and N. G. Kolosova*

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny,* Vol. 146, No. 10, pp. 435-438, October, 2008
Original article submitted April 2, 2008

OXYS rats are characterized by early development of cataract and chorioretinal degeneration with clinical manifestations similar to those observed in senile cataract and age-associated macular degeneration in humans. According to fundoscopy findings, the incidence of chorioretinal degeneration sharply increases in OXYS rats by the age of 4.5 months, when all animals develop signs of fundus oculi pathology. Morphological analysis of semithin sections of the posterior wall of the eye in OXYS rats aged 5 months showed that choroid vessels, pigmented epithelium, and radial glia were most vulnerable to injury. Retinal hypoxia and destruction of the pigmented epithelium associated with circulatory disorders in the choroid vessels presumably lead to injuries of the neurosensory cells (mainly the external segments) and a 3.5-fold increase in the percent of photoreceptors with nuclear pyknosis in comparison with the control. These results indicate that OXYS rats represent an adequate model of age-associated macular degeneration and can be used for studies of the pathogenesis of this condition and development of methods for its treatment and prevention.

Key Words: *chorioretinal degeneration; OXYS rats; age-associated macular degeneration*

Age-associated macular degeneration (AMD) is becoming the main cause of vision impairment and loss in elderly people in countries with well-developed economy [13]. According to various sources, 14-46% population aged over 65 years suffer from AMD in Russia [7]. The pathogenesis of AMD remains not quite clear, no effective treatments are available. One of the causes responsible for this situation is the polyetiological nature of the disease; the development of AMD depends on the effects and interactions of various genes and environmental factors. The pathogenesis of this condition is studied on biological models, but the development of AMD is usually associated with complex manifestations of aging and age-specific diseases.

Early aging SAM (P8) mice develop changes in the morphology of the fundus oculi (FO) similar to those observed in patients with AMD [12]. Complex manifestation of phenotypical signs of early aging is also characteristic of OXYS rats [3,6,8, 10,11,14]. These animals develop spontaneous cataract from the age of 1.5-2 months, the morbidity reaching 100% at the age of 4-6 months (*vs.* no more than 5% in Wistar rats). Comprehensive studies showed that lenticular changes in OXYS rats are similar to those observed in patients with senile cataract [3,4]. It was recently found that along with

Siberian State Medical University, Tomsk; *Institute of Cytology and Genetics, Siberian Division of Russian Academy of Sciences, Novosibirsk, Russia. ***Address for correspondence:*** kolosova@bionet.nsc.ru. N. G. Kolosova

cataract, OXYS rats develop FO changes clinically corresponding to AMD in humans [4,6].

The criteria of correspondence of the model to this or that disease include, in addition to clinical manifestations, the reaction to standard therapy and adequacy of changes at the morphological level. The two former criteria were confirmed [2,4,6,9], while morphological analysis of the chorioretinal complex was never carried out in OXYS rats.

We analyzed the FO status of OXYS rats of different age and the correlations between pathological manifestations at the clinical and morphological levels.

MATERIALS AND METHODS

The study was carried out on OXYS rats from Laboratory of Animal Breeding, Institute of Cytology and Genetics. Ophthalmoscopic examinations were carried out using a Betta direct ophthalmoscope after pupil dilatation by tropicamide. In order to evaluate the age-specific changes in FO, fundoscopy was carried out in 570 OXYS rats aged 6 weeks to 2 years. Wistar rats of the same age served as controls.

Animals for morphological studies of the main parameters of destruction in the retina were selected on the basis of fundoscopy findings in OXYS rats. The chorioretinal complex was evaluated in 10 OXYS rats aged 5 months; 10 Wistar rats of the same age served as controls. The eyes were resected under ether narcosis, the posterior wall of the eye was collected, fixed in 2.5% glutaraldehyde, postfixed in 2% OsO_4, dehydrated in ascending alcohols and acetone, and embedded in epon-812. Semithin sections were sliced on an LKB-4 ultratome and stained with 0.1% toluidine blue. The specific area of choroid vessels in the sections was evaluated using Avtandilov's ocular grid, number of layers and density of nuclei in the external nuclear layer were evaluated in the ocular frame at an area of 900 μ^2. Neurosensory cells (NSC) with nuclear pyknosis, pyknomorphic radial gliocytes, neurons in the internal nuclear and ganglion layers were counted.

The significance of differences in the means was evaluated using Mann—Whitney test. The differences were considered significant at $p<0.05$.

RESULTS

The first changes in FO of OXYS rats manifested from the age of 6 weeks; at the age of 3 months, 24% animals exhibited ophthalmoscopic signs characteristic of stage 1 retinal degeneration and 13% had signs of stage 2 condition. A sharp increase of the morbidity was characteristic of the age of 4.5 months, when pathological changes were detected in all animals. At this age, 75% OXYS rats presented with changes corresponding to the AMD predisciform stage and 25% had changes corresponding to the disciform stage. The disease progressed by the age of 6 months, when 30.5% OXYS rats developed the disciform and 2.9% animals had already the cicatricial stage of the disease. By the age of 12 months, all animals were ill, 44% of them had coarse irreversible changes in FO, 31% animals had the disciform stage of extensive hemorrhages and exudative detachment of pigmented and neuroepithelium of the retina. By the age of 24 months, the changes were irreversible in virtually all animals.

The first clinical signs of the disease in OXYS rats are redistribution of pigment, soft exudative foci of irregular shape with blurred contour, solitary petechial hemorrhages, signs of degeneration of the choriocapillary layer and pigmented epithelium. Progress into the disciform (exudative) stage is paralleled by the progress of edema in the central zone, enlargement of the zone of pigmented epithelium detachment, which eventuates in the neovascularization stage with exudative hemorrhagic detachment of the retinal pigmented epithelium and cicatrization. Even stenosis of the vessels is clearly discernible in OXYS rats with various clinical forms of degeneration. By contrast, only 7% Wistar rats developed primary pathological changes in FO by the age of 24 months.

Based on the results of FO examination, the animals with stable initial signs of chorioretinal degeneration corresponding to the AMD predisciform stage were selected for morphological studies at the age of 5 months. The findings indicate that at this age all structural elements of the chorioretinal complex of OXYS rats are involved into the pathological process, but the degree of involvement varied. Choroid vessels with thinned walls and stenosed lumen are subjected to significant destruction. Slugging of formed elements and thrombosis were observed in some vessels. The number of clotted vessels in OXYS rats was 19 times higher, while the number of patent vessels was 2.3 times less than in Wistar animals.

Changes in the pigmented epithelium were sharply degenerative. The majority of pigmented epitheliocytes decreased in size, their cytoplasm was sharply vacuolated, microvilli were destroyed, and basal plication disappeared. The specific area of pigmented epithelium was 34.4% decreased in comparison with the control.

Presumably, retinal hypoxia caused by circulatory disorders in the choroid vessels, destruction of the pigmented epithelium playing an important role

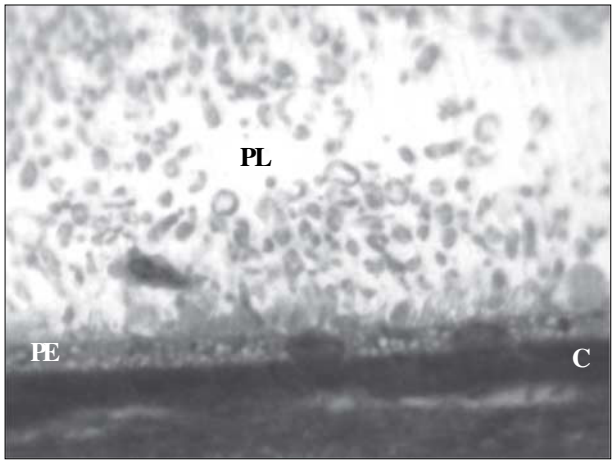

Fig. 1. Stratification and fragmentation of external segments of retinal photoreceptors in OXYS rats. Here and in Figs. 2, 3: Toluidine Blue staining, ×900. PE: pigmented epithelium; PL: photosensory layer; C: choroid.

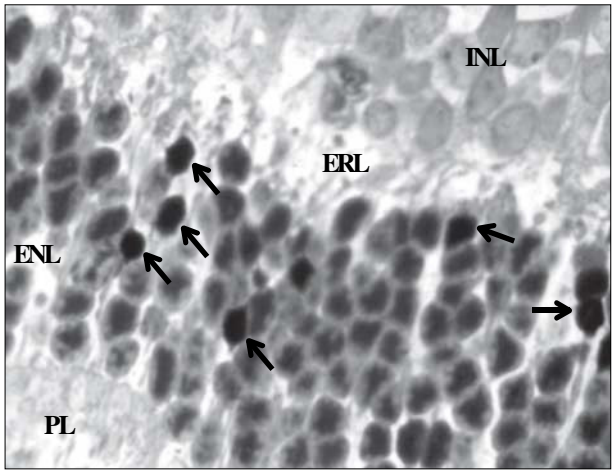

Fig. 2. Pyknosis of NSC nuclei in external nuclear layer of the retina in OXYS rats. Arrows show NSC pyknotic nuclei; PL: photosensory layer; ENL: external nuclear layer; ERL: external retinal layer; INL: internal nuclear layer.

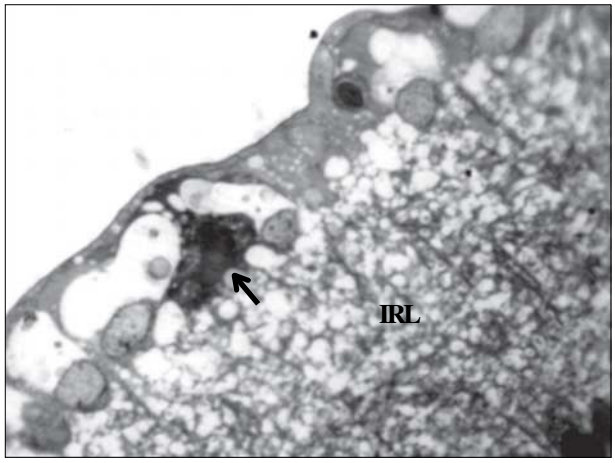

Fig. 3. Pyknomorphic ganglion neuron of the OXYS rat retina. Arrow shows pyknomorphic ganglion neuron; IRL: internal retinal layer.

in the antiradical and antihypoxic protection of the retina, and high sensitivity to oxidative stress, characteristic of OXYS rats [5], lead to NSC damage. This manifests mainly in injuries to the external segments (stratification, fragmentation, loss of connection to pigmentoepitheliocytes; Fig. 1). Changes in the nuclear part of NSC are less pronounced. The external nuclear layer of OXYS rats consists of 7-8 layers of NSC nuclei (*vs.* 12-13 layers in Wistar rats). Some nuclei are shifted to the photosensory and external retinal layers; scleral processes of the radial glia proliferate. Cells with nuclear pyknosis and perikaryon edema are seen (Fig. 2). Quantitative analysis showed high percentage of the pyknotic photoreceptor nuclei in OXYS rats (1.41± 0.32% *vs.* 0.40±0.02% in Wistar rats; $p<0.05$), while the numerical density of NSC nuclei in the external nuclear layer was 25.7% decreased (39,601±257 in Wistar rats; $p<0.05$).

The involvement of associative neurons depends on the NSC and radial glia status. Radial gliocytes of the retina undergo degenerative changes, which is seen from the increase in the percentage of pyknomorphic radial gliocytes from 1.10± 0.09% in the control to 8.50±1.49% in OXYS rats ($p<0.05$). The amacrine and bipolar neurons were most sensitive of the associative neurons in the OXYS rat retina. Neurons with destructive changes in the nucleus and cytoplasmatic edema were seen. The percentage of pyknomorphic bipolar and amacrine associative neurons in OXYS rats reached 2.0±0.5 and 1.46±0.39%, respectively, *vs.* 0.80± 0.08 and 1.00±0.12% in the control ($p<0.05$).

Multipolar neurons of the retina are characterized by markedly chromatophilic substance and high density of organelles. That is why the structural changes in the ganglion neurons in case of injury are evaluated by the percentage of cells with focal and total chromatolysis reflecting the distribution of chromatophilic substance in perikaryons, and the percentage of ganglion neurons modified by the dark type (with increased electron density of the perikaryon and with nuclear pyknosis; Fig. 3). Quantitative analysis showed that all these parameters were high in the retinal ganglion neurons of OXYS rats, which could be caused by hypoxia and disorders in the ganglion neuron metabolism.

Hence, the results demonstrated that FO changes in OXYS rats at the morphological and ophthalmoscopic levels were in good agreement. The choroid vessels, pigmented epithelium, and radial glia were most sensitive to injury. The neuron resistance in the internal layers can be due to compensatory opening and dilatation of intraretinal vessels in the external, internal retinal, and ganglion

layers. Changes in the chorioretinal complex of OXYS rats is a typical reaction to chronic hypoxia, one of the leading factors in the pathogenesis of AMD. Previously detected sclerotic changes in the cerebral vessels and cerebral circulation disorders in 12-month-old OXYS rats [1] confirm the vascular etiology of AMD. On the whole, the results of this study confirm that OXYS rats correspond to AMD model and can be used for studies of pathogenesis of this disease and development of therapeutic and preventive methods.

REFERENCES

1. I. G. Agafonova, N. G. Kolosova, N. P. Mishchenko, *et al.*, *Byull. Eksp. Biol. Med.*, **143**, No. 4, 446-450 (2007).
2. O. G. Gusarevich, A. Zh. Fursova, and N. G. Kolosova, *Klin. Oftalmol.*, **4**, 186-190 (2003).
3. N. G. Kolosova, P. A. Lebedev, S. V. Aidagulova, and T. S. Morozkova, *Byull. Eksp. Biol. Med.*, **133**, No. 10, 235-240 (2003).
4. N. G. Kolosova, P. A. Lebedev, A. Zh. Fursova, *et al.*, *Uspekhi Gerontol.*, **12**, 143-148 (2003).
5. N. G. Kolosova, N. A. Trofimova, T. V. Shcheglova, and S. V. Sergeeva, *Byull. Eksp. Biol. Med.*, **139**, No. 4, 387-390 (2005).
6. N. G. Kolosova, A. Zh. Fursova, P. A. Lebedev, and O. G. Gusarevich, *Oftalmol. Zh.*, No. 3, 237-242 (2004).
7. E. S. Libman and E. V. Shakhova, *Vestn. Oftalmol.*, No. 1, 32-35 (2006).
8. A. Zh. Fursova, O. G. Gusarevich, A. M. Gonchar, *et al.*, *Uspekhi Gerontol.*, No. 16, 76-79 (2005).
9. T. A. Shteklina, N. G. Kolosova, and A. Ya. Terner, *Ros. Fiziol. Zh.*, **91**, No. 10, 1213-1220 (2005).
10. A. A. Bobko, S. V. Sergeeva, E. G. Bagryanskaya, *et al.*, *Biochem. Biophys. Res. Commun.*, **330**, No. 2, 367-370 (2005).
11. N. G. Kolosova, T. V. Shcheglova, S. V. Sergeeva, and L. V. Loskutova, *Neurobiol. Aging*, **27**, No. 9, 1289-1297 (2006).
12. A. B. Majji, J. Cao, K. Y. Chang, *et al.*, *Invest. Ophthalmol Vis. Sci.*, **41**, No. 12, 3936-3942 (2000).
13. W. F. Schrader, *Ophthalmologe*, **103**, No. 9, 742-748 (2006).
14. S. Sergeeva, E. Bagryanskaya, E. Korbolina, and N. Kolosova, *Exp. Gerontol.*, **41**, No. 2, 141-150 (2006).