FOCAL CHORIORETINAL ATROPHY IN RATS

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Abstract: Focal chorioretinal atrophy observed in untreated Sprague-Dawley rats was clinically and histopathologically described. On ophthalmoscopic examination, the lesion consisted of discrete, hyperreflective, frequently rectangular foci with sharp margins. Petechial hemorrhages preceded to the development of these foci. No particular predilection sites were evident in the fundus. Histopathological characteristics on the perfusion-fixed eyes included: 1) the affected region was focal and well demarcated, 2) the retina in the lesion was devoid of the external layers, 3) in contrast, the internal retinal layers were unaffected, 4) abrupt disappearance of the underlying retinal pigment epithelium and choroid capillaries coincides with the retinal change, and 4) very little inflammatory cell response. These findings suggest that the primary defect may be either in the retinal pigment epithelium or choroid capillaries with subsequent retinal atrophy. (*J Toxicol Pathol* 6: 205~211, 1993)

Key words: Retina, Retinal pigment epithelium, Choroid, Atrophy, Rats

Introduction

Retinal atrophy is known to occur in laboratory albino rats. Morphologically these retinal atrophy may be classified into 2 groups, either generalized or focal. The generalized type is exemplified by hereditary retinopathy¹⁻⁴ and age-associated retinal degeneration⁴⁻⁶, and the focal type by the early stage of phototoxic retinopathy^{7,8}. Although some of these retinal changes have been extensively studied, the focal retinal atrophy sporadically observed in rats has received less attention. The involvement of regional choroid has not been recognized in spite of wide usage of rats in industrial toxicity testing. Because the focal chorioretinal atrophy (FCRA) described occurs in young rats, it has to be characterized in detail as a background lesion to enable differentiation from compound-induced changes in safety assessment studies.

Clinical and morphological features of FCRA found in untreated Sprague-Dawley rats are presented.

Materials and Methods

1. Animals

A total of 1853 male and 1924 female Crj: CD (SD) rats obtained from Charles River Japan Inc., Atsugi Breeding Center, Kanagawa, were individually housed in suspended wire-mesh cages and maintained in climate-controlled animal rooms with ambient temperature of $22 \pm 2^\circ\text{C}$, relative humidity of $55 \pm 10\%$ and a 12-hour on/off cyclic light. All rats had free access to tap water and balanced rodent chow (CE-2, CLEA Japan Inc., CRF-1, Oriental Yeast Co., Ltd. or 5002, Purina Mills). At the time of ophthalmic examination, rats ranged from 5 to 78 weeks old.

2. Ophthalmic examination

Prior to examination, a drop of mydriatic agent (Mydrin P, Santen) was applied to each eye. When the pupil was dilated, the posterior segment of the eye including the vitreous, retina and choroid

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was examined by indirect ophthalmoscopy (Neitz IO-H and 28 diopter aspheric lens). Fundus photographs were taken using a Kowa SL-2 camera.

3. Histopathology

Selected rats with fundus abnormalities were perfused through the heart with a combined fixative of 1% formalin and 1.5% glutaraldehyde in phosphate buffer solution of pH 7.2 under

pentobarbiturate anesthesia. The eyes were removed, post-fixed in the combined fixative overnight and processed to paraffin-embedded sections in a standard manner. The sections were cut at 5 μ m and stained with hematoxylin and eosin.

Results

The overall incidence was 0.5% and there was no intimate correlation with sex or age. Clinical-

Fig. 1. Normal rat retina. The RPE is continuous throughout the retina (arrow heads), and the retinal capillary beds (small arrows) and choroid capillaries (large arrows) are patent. Crj: CD (SD) Female, 5 weeks old. HE $\times$ 304.

Fig. 2. Section from focus "a" in Case 1. The left half of the retina is normal with continuous RPE (arrow heads) and patent choroid (large arrows). In the right half, the retina is devoid of the external layers. Absence of subjacent PRE and of choroid coincides with the retinal lesion. The retinal capillaries are patent (small arrows). A large macrophage (*) is seen in the subretinal space. HE $\times$ 304.

Plate 1. A: A normal rat fundus. The optic disc and holangioretinal vessels are shown. Crj: CD (SD) Female, 5 weeks old. B: Case 1. Fundus with FCRA (a and b). The lesions are hyperreflective and well-demarcated from surrounding normal fundus. Crj: CD (SD) Male, 24 weeks old. C: Case 2. Fundus with FCRA. The lesion is rectangular with sharp linear margin (arrow heads). Crj: CD (SD) Female, 8 weeks old. D: Case 3. Fundus with multifocal petechial hemorrhages (arrows). Crj: CD (SD) Female, 5 weeks old. E: The same fundus as in Plate 1-D, 24 hrs later. The regional fundus became hyperreflective creating a rectangular lesion (arrow heads). Petechial hemorrhages are aligned along the border of normal fundus (arrows). F: The same fundus as in Plate 1-D, 6 days later. The rectangular FCRA is formed (arrow heads). Small petechial hemorrhages are no longer seen.

Fig. 3. Section from "b" in Case 1. The retina in the lesion is herniated into the level of the choroid where cells in the inner nuclear layer are dispersed. These dislocated cells are accompanied by patent retinal capillaries (small arrows). RPE (arrow heads) and choroid (large arrows) in the left half are unaffected. HE $\times 304$.

Fig. 4. Section from Case 3. In sharp contrast to the normal retina (left half: RPE; Arrow heads, patent choroid; large arrows), the lesion is composed of decreases in number of cells in the outer nuclear layer, abrupt discontinuity of underlying RPE, and abrupt disappearance of choroid capillaries. The internal retinal layers including retinal capillaries (small arrows) are intact. The borderline between FCRA and normal retina is distinct as in Case 1. HE $\times 304$.

ly, FCRA was noted as discrete hyperreflective zones sharply outlined from surrounding normal fundus (Case 1, Plate 1-B). The lesions were frequently rectangular (Case 2, Plate 1-C). No clear predilection site for these lesions was evident in the fundus. There was no uniformity either in size or direction of the long axes; *i.e.* in some cases, the lesions were radially oriented from the optic disc and in other cases, they were arranged haphazardly across the fundus. Most cases were unilateral with bilateral lesions being very infrequent.

In several cases, the lesions were preceded by hemorrhage in the fundus. For example, a 5 weeks old female rat had multifocal petechial hemorrhages on the left fundus (Case 3, Plate 1-D). After 24 hours, this region of the fundus became hyperreflective. The hemorrhages were

aligned along the border line at this stage (Plate 1-E). Six days after hemorrhages were found, a rectangular hyperreflective zone became more apparent and small petechial hemorrhages were no longer evident (Plate 1-F).

Perfusion fixation well preserved the retinal and choroidal architecture with each retinal layer being clearly discernible. The retinal vessels, capillary beds distributed on each side of the inner nuclear layer, and choroidal capillaries are patent (Fig. 1).

Histological characteristics of the hyperreflective fundus are specific. In case 1, the retina is abruptly thinned in the region. The external retinal layers consisting of the visual cells and photoreceptors are completely absent in the atrophic retina. In sharp contrast, the internal retinal

layers are intact. The abrupt disappearance of the subjacent retinal pigment epithelium (RPE) and choroid capillaries coincides with the retinal changes (Fig. 2-4). Occasional large macrophages are present within the atrophic external retina (Fig. 2). Cells of the inner nuclear layer are occasionally herniated into the choroidal zone, and are accompanied by patent retinal capillaries (Fig. 3). In case 3, histological features may represent earlier stage to those observed in Case 1. The lesion is well delineated from adjacent normal tissues, and composed of coexistence of decreases in number of the outer nuclear layer and complete lack of underlying RPE and choroid capillaries. Yet, as in case 1, the internal retinal layers have no remarkable changes and retinal capillaries are patent (Fig. 4). Some macrophages located in the affected region contain hemosiderin pigment indicating previous hemorrhage.

Discussion

The FCRA reported here has the following characteristics; 1) petechial hemorrhages in the early stage, 2) rapid progression to hyperreflective foci in the fundus, 3) very sharp linear demarcation from surrounding normal fundus, 4) no predilection sites, 5) abrupt lack of the external retinal layers and intact internal retinal layers, and 6) abrupt lack of RPE and choroid capillaries which coincides with the area of retinal lesion.

Phototoxic retinopathy is not an uncommon lesion in albino rats^{4,7-12}, and is induced by prolonged exposure to light. Noell has classified these retinal changes into 2 types⁸. The type 1 is irreversible, and morphologically characterized by absence of the RPE and external retinal layers; whereas, the type 2 is reversible and the RPE remains intact with lack of the overlying external retinal layers. In both types, the choroid shows no abnormalities^{8,12}. FCRA clearly differs from phototoxic retinopathy in that the foci in FCRA are always devoid of normal choroid. Furthermore, to induce the type 1 phototoxic retinopathy where the RPE is lacking, prolonged exposure to intense light is required, which is unlikely to happen in standard animal facilities. The early onset and random fundic distribution of FCRA as

well as cage location of animals also exclude the possible association of these 2 retinal changes.

Retinal dysplasia is another focal retinal lesion^{13,14}. The retina has inward folds with disarranged retinal layers and occasional roset-like structures. Macrophages may be present within the foci. The border line of lesions are distinct. The dysplastic lesion becomes, unlike FCRA, thicker toward the vitreous and the RPE is continuous.

Age-related retinal degeneration has been reported in albino rats⁴⁻⁶. Although the change is most prominent histologically in the peripheral retina⁵, morphometric analysis revealed that the rate of cell loss in the inner and outer nuclear layers were similar at the central, midperipheral and peripheral retina⁶. Therefore, the age-related atrophic change occurs in diffuse manner, and the margin between the lesion and apparently unaffected retina is poorly defined showing gradual transition. Based on the generalized distribution, and ophthalmoscopic and histological characteristics, the age-related retinal change is easily distinguishable from FCRA. In addition, the RPE is continuous in the retina with age-related changes unlike in FCRA. The onset of the FCRA, as early as 6 weeks old, is also inconsistent with age-related retinal degeneration.

The pathogenesis of FCRA is not certain at the present. Infectious origin is most unlikely since there is no evidence of an inflammatory reaction.

The acute and invariably focal nature together with involvement of the external retinal layers, RPE and choroid may suggest a disturbance in the retina-RPE-choroid complex. The internal retinal layers are supplied by the retinal circulation consisting of the retinal arteries/veins and 3 capillary beds that lie at the levels of nerve fiber layer and both sides of inner nuclear layer, respectively. On the other hand, the external retinal layers are avascular and receive nutrient primarily from choroidal circulation through the RPE¹⁵. These differences in vascular supply may account for unique features of FCRA. It is noteworthy that in FCRA the internal retinal layers are always spared and retinal capillaries are patent even in a case where the inner nuclear cells are herniated

into the level of the choroid. The preserved retinal vessels appear to supply these cells in the internal retinal layers. In contrast, lack of choroid could affect the blood supply to the dependent tissues, which are the RPE and external retinal layers, resulting in regional ischemia and subsequent atrophy. Indeed, this mechanism is proposed in an other form of chorioretinal degeneration¹⁶. However, findings that may explain possible vasculo-occlusive pathology or any vasculopathy were not observed in our material. Examination of vascular cast indicated complicated capillary networks in the rat choroid^{12,17}. It would seem unlikely that the sharp linear margin could be drawn in such a dense capillary complex even if one or several arterioles are obstructed. More detail observation of the choroidal vasculature would be required to obtain concrete evidence for supporting this hypothesis.

The physiological interrelationship in the retina-RPE-choroid complex is not simple and has been a subject for investigation¹⁸. The RPE is essential to maintain retinal function and to support a repair process of visual cell outersegments^{19,20}. The RPE influences the development of the choroid²¹. Recently, it was suggested that the RPE modulates the function and structure of the underlying choroid in mature eyes whereby dysfunction of the RPE is followed by degeneration and atrophy of local choroidal capillaries^{17,18,22,23}. In FCRA, the RPE is discontinuous within the lesion. However, no definitive conclusions as to the onset of RPE disintegration could be made from the present study since it is uncertain whether the RPE or choroid first undergoes degeneration and atrophy. Lin *et al.* described electron-microscopically the retinal degeneration in rats and classified it as mild, intermediate and severe stages²⁴. They showed RPE alteration starting in the mild stage. This finding may suggest that the earliest sequence of events takes place in the PRE though the exact time relationship between the RPE and choroidal degeneration has yet to be elucidated.

Thus, the FCRA in albino rats has several distinctive features. It is unlikely retina-oriented disease, but the cardinal defect seems to reside either in the RPE or choroidal capillaries leading

to regional retinal atrophy. Infectious and environmental factors do not appear to be the primary cause, and the involvement of other influence such as genetic problems have to be considered.

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