Morphological Changes of the Sensory Retina Following the Occlusion of Ciliary Arteries in Rabbits

Dong-Seob Kim, M.D., Myung-Kyoo Ko, M.D., and Joon Kiu Choe, M.D.

Department of Ophthalmology, Hanyang University Hospital, Seoul, Korea

The whole retina, except for the medullary fiber zone in a rabbit eye, is supplied by choroidal circulation. Therefore, the histopathological changes of the sensory retina due to choroidal circulatory disturbance in rabbits may be comparable to that of the human sensory retina in the case of ophthalmic artery occlusion.

This study was carried out to evaluate the histopathological changes of the ischemic retina secondary to the occlusion of choroidal circulation. The experimental occlusion of all posterior ciliary arteries and anterior ciliary arteries in the horizontal rectus muscle of rabbit eyes was performed and the subsequent histopathological changes of the sensory retina were observed by transmission electron microscopy.

The morphological changes of the sensory retina following the occlusion of the ciliary arterial system are as follows: severe loss of the inner and outer segments of the photoreceptor, mild to moderate degeneration of the ganglion cells, and excellent preservation of the Müller’s cell fibers and the extension of the cytoplasmic villous processes to the cytoplasmic vacuolar spaces of other degenerated cells.

These findings indicate that the Müller’s fibers in the ischemic condition of retina might contribute to the formation of gliosis or scarring of a damaged retina.

Key words: Müller cell, occlusion of ciliary artery, retina, ultrastructure

INTRODUCTION

It is well known that the human retina has two sources of blood supply. The outer layer of the sensory retina and the fovea centralis receive their blood supply from the choroidal circulation, and the inner layer from central retinal circulation. Disorder of the choroidal perfusion can lead to the disturbance of the outer layer of the retina involving photoreceptor cells as well as retinal pigment epithelium (RPE). Clinically, retinal lesion can be influenced by abnormal choroidal circulation and in such cases, the understanding of the ciliary circulatory system is essential to interpreting retinal disorders relating to choroidal circulation.

This study was performed to evaluate the histopathological changes of the sensory retina secondary to the occlusion of the choroidal circulatory system, which were frequently observed clinically. The vascular occlusions in rabbits were performed experimentally at the long posterior ciliary artery (PCA), the short PCA, and the anterior ciliary artery (ACA) in the horizontal rectus muscle to prevent possible retrograde filling.
MATERIALS AND METHODS

Occlusion of Ciliary Arteries

After retrobulbar anesthesia with 0.5 ml of 1% lidocaine in five black adult rabbits, a lateral canthotomy was done to expose the posterior part of the eyeball. The optic nerve in the orbit was tied with 4-0 black silk to block possible circulation of the posterior ciliary arteries adjacent to the optic nerve. The long and short PCA were cut using a No. 11 blade to produce an artificial occlusion of the PCA system at the scleral level. We also tied the medial and lateral rectus muscles with 4-0 black silk at their insertion site to prevent possible retrograde filling of the anterior part of the choroid which is supplied by the recurrent artery of the ACA.

A slit-lamp examination was performed every other day for a week. A fundus examination, however, was impossible from the day after the above procedure due to corneal edema, Descemet’s folding of the cornea and inflammation in the anterior chamber. The eyes were enucleated 3 weeks after the experimental occlusion of the ciliary arterial system.

Electron Microscopic Examination

After enucleation, the eyes were divided in half at the equator level and the vitreous body was removed. The sensory retina obtained near the medullary fiber zone was fixed in glutaraldehyde and postfixed in osmium tetroxide, dehydrated in ethyl alcohol, and embedded in Epon. The tissue was then sectioned at 1 μm thickness with a tissue microtome, and after staining with toluidine blue, sections were selected to study with an electron microscopy. Transmission electron microscopy (Hitachi-600, Japan) was performed after double staining with uranyl acetate and lead citrate.

RESULTS

The outer retinal layer showed the villous cellular processes of Müller’s cell beyond the external limiting membrane and some RPE cells exhibited phagosome complex within their cytoplasm. In some areas of the outer retina, there was a patchy

Fig. 1. Three weeks after the ciliary artery occlusion. An electron micrograph of the outer retina showing the villous cellular processes of the Müller cell beyond the external limiting membrane and the phagosome complex of retinal pigment epithelium (arrow) (× 5,000).

loss of the external limiting membrane with infiltration of mononuclear cells and fibrin network. Some phagosome complexes were also noted. In other areas of the outer retina, an extensive loss of inner and outer segments of photoreceptor cells, infiltration of fibrin and mononuclear cells in subretinal space, and prominent villous processes of the Müller’s cell were observed (Fig.1).

In the outer nuclear layer, the outer plexiform layer, the inner nuclear layer, and the inner plexiform layer, the degenerative change and

Fig. 2. Three weeks after the ciliary artery occlusion. An electron micrograph of the outer retina showing the condensation of the nuclear chromatin (arrows) of the outer layer of the retina (× 4,000).
Fig. 3. Three weeks after the ciliary artery occlusion. An electron micrograph of the inner layer of the retina showing the infiltration of mononuclear cells ($\times$ 4,000).

Fig. 4. Three weeks after the ciliary artery occlusion. An electron micrograph of the inner layer of the retina showing the vacuolation of the axon ($\times$ 3,000).

Vacuolation of the cytoplasm were observed with the condensation of the nuclear chromatin and nuclear pyknosis, which made identification of the cellular microorganelles difficult (Fig.2). The inner retinal layer also showed infiltration of mononuclear cells (Fig.3), and marked vacuolation of the axon (Fig. 4).

Mild cellular changes were noted in the ganglion cells of the inner layer of retina, such as condensation of the nuclear chromatin, and pyknosis of the nucleus with a relatively well-preserved rough endoplasmic reticulum within the cytoplasm (Fig.5). The internal limiting membrane was intact but the adjacent nerve fibers showed vacuolation of the cytoplasm and a well-preserved Müller’s cell, which extended numerous villous processes to the cytoplasmic vacuolar spaces of the nerve fiber axons (Fig.6).

DISCUSSION

The short PCA mainly contributes to the choroidal circulatory system. Some degree of the anterior choroidal circulation is derived from the recurrent branches from the major arterial circle of the iris, which are composed of branches from the temporal and the nasal long PCA as well as from the ACA. In choroidal circulation, there are anatomical interconnections between the arteries, venules, and choriocapillaries, respectively, but functionally they act as a terminal branch of the
Fig. 6. Three weeks after the ciliary artery occlusion. An electron micrograph of the inner layer of the retina showing the numerous villous processes of the Muller cell through the cytoplasmic vacular space. Note the intact internal limiting membrane (× 6,000).

corresponding sector. Therefore, in the ischemic condition of choroidal circulation, a triangular syndrome or Fuch’s spot can occur in the corresponding watershed zone, especially when there are no retrograde fillings of the ischemic area. As the choroidal circulation supplies the outer half of the sensory retina, triangular syndrome is a chorioretinal scar induced by sectorial choroidal ischemia and corresponds to the nonperfused territory of a PCA.

As mentioned above, the anterior part of the choroid is supplied by the recurrent artery, therefore we produced a total experimental occlusion of the choroidal circulation in a rabbit eye by the interruption of all arterial supply of long and short PCA and ACA in the horizontal rectus muscle. This might prevent possible retrograde filling from the adjacent area of the ischemic zone. Because the vascular supply of the anterior portion of the eye is compromised, anterior segment ischemia can occur following either strabismus surgery or retinal detachment surgery with disinsertion of the rectus muscles. The rabbit eye in this study showed cells and flares in the anterior chamber and Descemet’s folds a day after the experimental occlusion.

The ultrastructural findings in this study revealed that the inner and outer segments of the photoreceptor cells were completely lost, but the zonular adherenses connecting the Muller’s cell and the photoreceptor cell were intact in some areas. In the ischemic condition of the choroidal circulation, the inner and outer segments of the photoreceptors were most susceptible, and destruction of the external limiting membrane and infiltration of the fibrin network and mononuclear cells were also noted. In a rabbit eye, retinal vessels supply only the medullary fiber zone of the retina. We selected the retina just adjacent to the medullary fiber area, which has neither retinal vessels nor a blood retinal barrier in the selected zone.

Therefore, subretinal and intraretinal infiltration of the fibrin and the mononuclear cells may reflect the destruction of the barrier function between RPE and Bruch’s membrane under ischemic insult of the choroid.

We also observed morphological changes through the inner layer of the retina. Cellular degeneration such as the condensation of the nuclear chromatin, pyknosis of the nucleus, and cellular edema with vacuolation were noted in the inner retinal layer. Ganglion cells exhibited relatively mild changes and showed well-preserved rough endoplasmic reticulum within the cytoplasm. Retinal nerve fibers also revealed degenerative changes, but Muller’s fiber had well preserved cytoplasmic configurations through the entire retinal layer and extended cytoplasmic villous processes to the space of the degenerated axons of other cells. Muller’s cell, therefore, can be considered to be the most resistant to ischemic insults.

In contrast to the human retina, which has two sources of blood supply from the ophthalmic artery, the first being the retinal arterial system supplying the inner half, and the second being the ciliary arterial system supplying outer half, the whole retina of the rabbit eye is supplied by choroidal circulation, except for the medullary fiber zone. Therefore, deficient choroidal perfusion in a rabbit eye can be comparable to disturbance of the ophthalmic arterial perfusion in the human eye.

In the ischemic condition of the rabbit choroidal circulation, the degeneration of the inner and outer segments of the photoreceptor occurred earlier, and the degeneration of the ganglion cell was less severe and its cytoplasmic configuration relatively
preserved. The Müller's cell was the most resistant to the ischemic insult and preserved its morphological characteristics even in the late stage. These histopathological results provide the supporting evidence that the Müller's fiber in the ischemic condition plays an important role in gliosis or scarring of the retina.

REFERENCES


