Experimental Central Serous Chorioretinopathy

IV : Fluorescein Angiography and Electron Microscopy During Spontaneous Healing Process

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Summary: Spontaneous healing processes of experimental central serous chorioretinopathy induced by repeated intravenous adrenalin injections were studied in detail by fluorescein angiography and electron microscopy. The abnormal fluorescein spot in this experimental model changed from the initial ink blot type into the residual type and, further, into the window defect type, before finally disappearing. These findings indicate that the healing process in our model closely resembles those in human. Further, the histological findings suggest that the spot of either residual type or window defect type identified by fluorescein angiography is due to marked enlargement of basal infoldings of pigment epithelial cells and separation of the intercellular gap junction between the pigment epithelial cells; furthermore, the difference between the two types of fluorescein spots depends on the difference in the degree of such morphological changes, mentioned above.

Key words: experimental central serous chorioretinopathy — Japanese Monkey — electron microscopy — fluorescein angiography — spontaneous healing — basal infolding — intercellular gap junction —

Introduction

We have produced an animal model which closely resembled human central serous chorioretinopathy in monkey by repeated intravenous injections of adrenalin and have reported the associated fluorescein angiographic findings of the initial stage of the onset (Yoshioka et al. 1981 a, b, c; Yoshioka et al. 1982) and electron microscopic appearance at leakage sites of fluorescein (Yoshioka and Katsume, 1982 a, b,).

The main purpose of this study was to establish whether experimental central serous chorioretinopathy in monkey eyes develops in similar manner to that in hu-

man eyes, with respect to fluorescein angiographic changes, even after its onset.

We also aimed to describe histopathological features during the spontaneous healing process at the sites of hyperfluorescence in the macular area.

Materials and Methods

In this study, two Japanese monkeys and two cynomolgus monkeys were used; one of Japanese monkeys (5 years old, 5 kg in weight) with multiple leakage spots of fluorescein was selected because the presence of a sufficiently large number of spots was thought to facilitate the light

microscopic examination of the lesion.

Adrenalin was repeatedly injected intravenously, essentially by the same method as previously reported (Yoshioka et al. 1981 a, b, c; Yoshioka et al. 1982; Yoshioka and Katsume, 1982 a, b): Adrenalin was injected intravenously once a day at about the same time of day at the initial dose of 0.167 mg/kg for the first 7 days, increasing to 0.33 mg/kg from the 8th to 14th day and, from the 15th day, the dose was standardized at 0.5 mg/kg. As in previous experiments, adrenalin was administered every day but, even if interrupted for unavoidable reasons, the interval between doses was never allowed to exceed 3 days. Monkey eyes were regularly examined by ophthalmoscopy combined, whenever necessary, by color fundus photography and fluorescein angiography. Especially in this experiment, more frequent observations were made on the course of development after the onset by color fundus photography and fluorescein angiography. Mydriasis was achieved with 1 % atropine instilled into the conjunctiva. A dose of 0.5 ml/kg (i. v.) of 10 % sodium fluorescein was used for fluorescein angiography. General anesthesia was provided with an intramuscular injection of ketamine hydrochloride (Sankyo, Co., Tokyo). A Topcon TRC-F3 fundus camera was used for color fundus photography and fluorescein angiography.

The absence of any pre-existing abnormality in the fundus of the monkey was confirmed by color fundus photography and fluorescein angiography.

Throughout the entire course of this experiment, the animal showed neither any side-effect of sodium fluorescein such as vomiting, *etc.* nor other abnormalities.

In preparation of specimens for transmission electron microscopy, after thoracotomy under general anesthesia, 2 % glutaraldehyde-2.5 % paraformaldehyde in 0.1 M phosphate buffer (pH 7.4) was perfused from the left cardiac ventricle for

fixation. Both eyes were then enucleated and dissected anteroposteriorly through the equator into 2 sections. Under direct microscopic observation of the posterior pole, the sites of abnormal fluorescein angiographic findings (shown in Fig. 3) were excised.

Excised samples were immersed in the same ice-cooled fixative for 2 hours. After washing in 0.1 M phosphate buffer (pH 7.4) for 30 minutes, the prefixed samples were refixed in 2 % osmium tetroxide in the buffer (pH 7.4) (ice-cold) for 1 hour, rapidly dehydrated in graded concentrations of acetone and embedded in epoxy resins. The embedded sections were sliced into serial sections in 1-2 μ m thick using a MT-1 or MT-2B ultramicrotome (Porter-Blum). After staining by toluidine blue, the site of observation was fixed under light microscopic view. The adjacent areas were then sliced into ultrathin sections using the same ultramicrotome and stained with lead hydroxide and uranyl acetate for observation with a Hitachi H 500 electron microscope.

Results

1. Clinical findings

Central serous chorioretinopathy occurred after the 42nd intravenous injection of adrenalin in this experiment. Figure 1 shows the fundus photographs of the right eye (A) and left eye (B), and fluorescein angiograms of the late phase (C and D) on the day of the onset. Localized disciform serous detachment of the retina was observed in the macular region of both eyes (Fig. 1, A and B, arrows). Fluorescein angiography revealed multiple leakage spots of fluorescein within the region of the detached retina in both eyes, and the leakage of the dye increased gradually (Fig. 1, C and D). Figure 2 (A-F) shows the fundus photographs and fluorescein angiograms in the same animal on the

following day, after the 43rd adrenalin injection. Localized disciform serous retinal detachment is markedly decreased in area and becomes milder. In the right eye, two detached regions are observed laterally and medio-inferiorly to the foveola (Fig. 2, A; arrows). In the left eye, round retinal detachment of an optic disc size was noted superiorly to the foveola (Fig. 2, B; arrows). Fluorescein angiography of the right eye disclosed fluorescein emerging as dots in the area of detached retina; some of them grew larger into concentric circles (Fig. 2, C and E). In the left eye, multiple fluorescein dots appeared in the macular region in the arterial phase (Fig. 2, D), and those within the area of the detachment grew into the leakage spots of ink blot type while those outside changed into hyperfluorescent spots in the venous phase (Fig. 2, F). Furthermore, on the 3rd day after the onset, *i. e.*, after the 45th adrenalin injection, disciform serous retinal detachment in the right eye disappeared without fluorescein leakage and the fluorescein dots in the macular region, seen in Fig. 3, A assumed the spots of window defect type with time (Fig. 3, C). In the left eye, serous retinal detachment still persisted and fluorescein angiography revealed the presence of one fluorescein leakage spot of ink blot type on the temporal side of the optic disc (Fig. 3, D; double arrows) and a few traces of subretinal fluorescein leakage in the latero-inferior region of the optic disc (Fig. 3, D, arrows). However, other fluorescein spots faded into the spots of the residual type with time (Fig. 3, B and D).

In this experiment as well as in the previous ones, abnormalities such as subretinal hemorrhage, retinal exudate and cystoid macular edema did not occur and fluorescein angiographic findings did not reveal either any changes in the optic disc or any disturbances in the retinal and choroidal circulation throughout the course of events.

2. Histological findings

Photographs shown in Figs. 5, 6 and 7 are the light and electron microscopic findings associated with abnormal fluorescein spots in the macular region of both eyes that were enucleated immediately after color fundus photography and fluorescein angiography taken after the 45th intravenous adrenalin injection.

A light microscopic view of the macula of the right eye, which appeared almost normal with no retinal detachment after spontaneous restoration of the pigment epithelium, is shown in Fig. 4, A; it shows, however, the sporadic presence of spaces at the basal region of cells (arrows). Fig. 4, B shows a light microscopic view of the macula of the left eye in which the sensory retina was detached from the pigment epithelial cell layer; the pigment epithelial cells were irregular in size and shape, producing spaces at the basal region of the cell and between adjacent cells (arrows).

Fig. 5 is an electron microscopic view of the pigment epithelial cells shown in Fig. 4, B. Basal infoldings of the pigment epithelial cells were remarkably enlarged to form wide spaces, but the intercellular junctional complex between the basal infoldings and the basal lamina of the pigment epithelial cell was preserved (vertical arrows). Furthermore, although separation of intercellular gap junction between the adjacent cells was noted, the tight junction was intact (horizontal double arrows). An increase of mitochondria was also noted within the cytoplasm.

On the other hand, the electron microscopic view of the pigment epithelial cells of the right eye shown in Fig. 4, A showed almost normal findings of the cells that have apical microvilli surrounding the rods and cones outer segments (Fig. 6). Despite slight separation of intercellular gap junction, the tight junction appears intact (Fig. 6, arrow). However, even in the same right eye, enlargement of basal in-

foldings and separation of the intercellular gap junction was still observed in the region shown by arrows in Fig. 4, A. Electron-dense particles were sporadically observed in Bruch's membrane and were indicative of a mild edematous change (Fig. 7).

Discussion

In several of our previous studies, we have reported that a pathology which corresponded ophthalmoscopically as well as fluorescein angiographically almost exactly to central serous chorioretinopathy in man, was induced in the posterior pole, mainly the macular region, of monkey eyes by repeated intravenous injections of adrenalin. Moreover, we have shown the usefulness of this experimental lesion as a model of this disease in man.

On the other hand, this disease is known to have a good prognosis and good visual acuity because of its spontaneous healing after the onset, the characteristic findings of the transition of fluorescein leakage patterns after the onset have been reported by ourselves. (Yoshioka et al., 1969; Yoshioka and Sugita, 1969), also by Watanabe and his colleagues. (Watanabe et al. 1969 a, b) and by many other investigators. In summary, the fluorescein leakage that appears in the initial phase of the onset in the form of the leakage spot of jet type or ink-blot type changes into the spot of residual type which persists in the same shape and size, even after disappearance of background choroidal fluorescence following the onset. Gradually, at the stage of healing as judged clinically, the fluorescein dot that appears in the arterial phase undergoes a change into the spot of windowdefect type that disappears with choroidal background fluorescence; finally these abnormal fluorescein spots disappear.

According to the present findings of

the (spontaneous) healing course followed by fluorescein angiography in our model of this disease, the onset of serous retinal detachment was confirmed after the 42nd intravenous injection of adrenalin and multiple leakage spots of ink-blot type were observed within the lesion. On the following day, serous retinal detachment seen in the macular region of both eyes was reduced, and the abnormal fluorescein leakage spots that had been of ink-blot type on the previous day remained unchanged in both size and shape notwithstanding the passage of time. After a further 2 days, serous retinal detachment of the right eye disappeared leaving only the spots of window defect type in the macula, as observed by fluorescein angiography. In the left eye, although the serous retinal detachment still persisted with the presence of the leakage spots of ink-blot type, other abnormal spots were changed to the spots of residual type.

In the previous experiments, we reported that serous retinal detachment showed spontaneous disappearance without any scar in the retina, irrespective of whether repeated adrenalin injections were discontinued or continued after the onset. Therefore, the present experiments confirmed such previous findings and verified that this experimental model closely corresponds to central serous chorioretinopathy in man, even with respect to the spontaneous healing processes.

Findings of interest were also obtained by histological observation of the regions corresponding to the spots of residual or window defect type. The abnormal findings obtained in this study from the light microscopic specimens were the formation of spaces at the basal region of the pigment epithelial cells and also between the pigment epithelial cells: these seem to correspond to the regions of the spots of residual or window defect type shown in Fig. 3. Electron microscopy of these sites revealed marked enlargement of basal in-

foldings of the pigment epithelial cells and separation of the intercellular gap junction between the adjacent cells.

Abnormal fluorescein leakage patterns of the residual and window defect types appearing in the healing stage of this disease are usually thought to suggest the presence of pigment epithelial cell detachment. Histologically, pigment epithelial cell detachment has been reported to be separation of intercellular junction between the basal infoldings and basal lamina of the pigment epithelial cell (Gass et al. 1966; Gass, 1977) or gap formation between the basal lamina of the pigment epithelial cell and the inner collagenous zone of Bruch's membrane (Yannuzzi et al. 1979; Coscas, 1980).

In spite of these postulations, however, abnormal findings such as pointed out were not detected in any of the regions of abnormal fluorescein spots in the present observation. Hence, the results obtained in this experiment suggest that the spot of residual and window defect type are not due to pigment epithelial cell detachment, but to serous retention within the region of markedly enlarged basal infoldings of pigment epithelial cell and the separated area of gap junction between the cells. Moreover, such abnormal findings common to the region of the spot of residual type found in both eyes and window defect type found in the right eye indicate that the histopathological differences between these 2 types of abnormal fluorescein spots do not represent qualitative but quantitative (degree of change) differences.

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Explanation of Figures

Fig. 1. Ophthalmoscopic and fluorescein angiographic findings of the right (A, C) and left (B, D) eyes after the 42nd intravenous adrenalin injection. Localized disciform serous retinal detachment was noted in macular region of both eyes (A and B, arrows). Fluorescein angiography revealed multiple fluorescein leakage spots in region of detached retina, presenting an ink-blot pattern in venous phase (C and D).

Fig. 2. Ophthalmoscopic and fluorescein angiographic findings of the right (A, C, E) and left (B, D, F) eyes after the 43rd intravenous adrenalin injection. Localized disciform serous retinal detachment in macular regions became reduced (A and B, arrows). Fluorescein angiography revealed decreased fluorescein leakage as dots in the region of detached retina of the right eye, and some of them grew into ink-blot pattern (C and D). In left eye, multiple leakage spots appear in arterial phase (C, D). In F, those in region of detached retina developed an ink-blot pattern and those outside the region changed into hyperfluorescent spots in the venous phase.

Fig. 3. Fluorescein angiographic findings after the 45th intravenous adrenalin injection. In the right eye, spots of fluorescein leakage (A) in the macula developed into spots of window defect type with time (C). In the left eye, some of the dotted spots of fluorescein leakage (B) that appeared in the arterial phase showed the leakage spots of ink-blot type (D, arrows), but others the spots of residual type (D).

Fig. 4. Light microscopic view of the lesion in the macula of the right (A) and left (B) eye. In the right eye, retinal detachment was not observed and despite restoration of the pigment epithelium in one layer, spaces were partially formed at the basal region of pigment epithelial cells (A, arrows). In the left eye, the sensory retina (SR) was detached from the pigment epithelial cell layer and the pigment epithelial cells were irregular in size and shape, forming spaces at the basal region of cells and between adjacent cells (B, arrows). Toluidine blue stain. A×70 B×180

Fig. 5. Electron microscopic view of Fig. 4, B. Marked enlargement of basal infoldings of the pigment epithelial cells was noted. Although separation of the intercellular gap junction was observed, separation of the tight junction was not present (double arrows). Cell contact was preserved between basal infoldings and basal lamina of pigment epithelial cells (vertical arrows). The number of mitochondria (M) was increased. ×8,500

Fig. 6. Electron microscopic view of Fig. 4, A. Pigment epithelial cells retained almost the normal morphology and had microvilli of the type that surrounds the outer segments (os) of rods and cones. Separation of intercellular tight junction was not observed. (arrow). ×5,700

Fig. 7. Electron microscopic view of the arrow-indicated area in Fig. 4, A. Enlargement of basal infoldings of the pigment epithelial cells and separation of intercellular gap junction were seen. Electron dense particles (EDP) are scattered in Bruch's membrane (BM).

×12,000