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STUDIES ON THE BLOOD-RETINAL BARRIER AFTER CRYORETINOPEXY WITH VITREOUS FLUOROPHOTOMETRY

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ABSTRACT

The extent of damage to the blood-retinal barrier, induced by cryoapplication on the sclera, was measured in adult pigmented rabbits by using a vitreous fluorophotometer. In eight isolated cryopexies, the intensity of each was equalized by stopping freezing exactly when a white spot started to appear in the fundus, and the blood-retinal barrier damage was repaired 7-10 days after the operation.

On the other hand, in the eyes treated with double cryoapplication refreezing being initiated just after the initial ice ball in the fundus disappeared, fluorescein concentration was reduced below the control value in 3-5 days but histopathological reparation of the retinal pigment epithelium was incomplete in these cases. The eyes treated with 20 scattered applications showed more profuse leaking of the fluorescein dye and required 35-42 days before the concentration of fluorescein was reduced below the control value; however, when the leaking ceased, the regenerated retinal pigment epithelium covered the Bruch's membrane. The above findings indicate that to prevent protraction of blood-retinal barrier damage associated with the retinal pigment epithelium, cryoretinopexy twice or more to the same area or extremely extensive cryoapplications at one time are prohibitive.

Keywords: Vitreous Fluorophotometry, Cryoretinopexy, Blood-Retinal Barrier, Pigmented Rabbits.

INTRODUCTION

Cryosurgery recently used for the treatment of retinal detachment has had the advantage of minimizing the histopathologic damage to the sclera on one hand, but on the other, the disadvantage of excess coagulation caused by repeated applications due to non-appearance of cryospots on the sclera.

The effect of cryosurgery on the blood-retinal barrier has been studied histopathologically by using horseradish peroxidase[1] or fluoroangiographically.[2] However, to our knowledge, quantitative analysis has not so far been attempted. This paper presents findings on the blood-retinal barrier by cryoretinopexy obtained by experiments with pigmented rabbits.

METHODS

Adult pigmented rabbits weighing 2.0-3.0 kg were used. Following pupillary dilatation with 5% phenylephrine hydrochloride and 0.5% tropicamide, topical anesthesia was provided with 0.4% benoxyle®. Under observation of the fundus through a binocular indirect ophthalmoscope, cryoretinopexy was performed. The absence of blood-retinal barrier damage had been preoperatively confirmed by vitreous fluorophotometry. The concentration of

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fluorescein in the mid-vitreous 60 min. after dye injection (less than 2.0×10^{-8}g/ml on the average) was used as a control value. Cryoapplication was performed under the following conditions:

1. It was applied to 8 regions in the mid-periphery of 8 eyes (8 rabbits) with the "medium" intensity defined by Lincoff[3] (coagulation was stopped immediately after the appearance of a white cryospot in the fundus). Each cryoapplication was made with care to avoid overlapping of white cryospots.
2. Eight double cryoapplications were performed in the same regions of 11 eyes (10 rabbits). The method used in this process was such that just after the initial ice ball in the fundus had disappeared, freezing was reinitiated on the same region to confirm the appearance of white cryospots.
3. Twenty scattered cryoapplications were performed in 8 eyes (4 rabbits).

At 60 min. after intraveneous injection of 50 mg/kg of 10% sodium fluorescein, its concentration in the mid-vitreous was measured. Fluorophotometry was repeated until the value dropped below the control value (2.0×10^{-8}g/ml). The vitreous fluorophotometer used was a photoslit-lamp microscope (Carl Zeiss, Jena) with an attached photomultiplier tube and amplifier. It recorded with an X-Y recorder, as described in the previous report.[4] The angles of the excitation light beam and detective field were fixed, respectively, at 15 degrees to each side of the sliding axis of the microscope. The detective field was 0.7 mm in diameter at magnification ×1 as previously reported.[4]

As the calibration system, a light source dimmer was used. The intensity of the light source was always kept constant with a photo-transducer (a silicone photocell) for standardization of the measurement.

RESULTS

Six of the 8 eyes that received ordinary cryoapplication showed their highest value of fluorescein concentration on the first day, ranging from 4.0×10^{-8} to 1.2×10^{-7}g/ml. In the remaining 2 eyes, the concentration peaked on the third day. The value dropped below the control on the seventh day in 7 eyes and on the tenth day in 1 eye (Table 1 and Fig. 1).

No. \ days	1	3	5	7	10
No. 1	4.0	9.0		2.0	1.0
No. 2	12.0	7.0		3.0	2.0
No. 3	4.0	3.0		1.0	1.0
No. 4	9.0	14.0	3.0	2.0	
No. 5	11.0	10.0	3.0	1.0	
No. 6	7.0	23.0	1.0	0	
No. 7	9.0	8.0	5.0	2.0	
No. 8	10.0	4.0	3.0	1.0	
Mean±S.D	8.3±3.0	9.8±6.4	3.0±1.4	1.7±0.8	1.3±0.6

Table 1. Fluorescein concentration in the mid-vitreous of the ordinary cryoapplication (g/ml × 10^{-8})

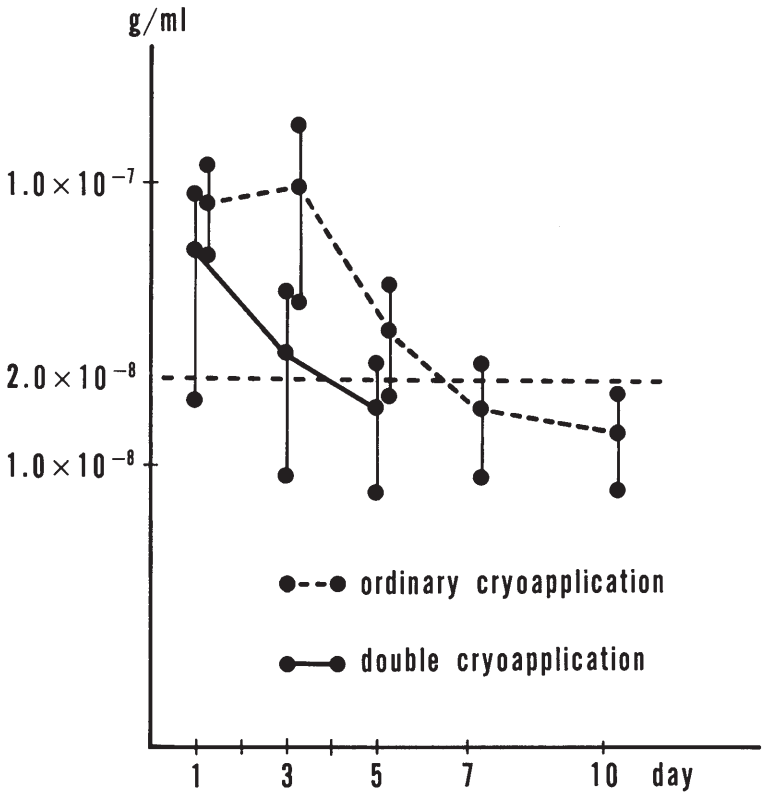

Fig. 1. The fluorescein concentration (mean ±SD) in the mid-vitreous after ordinary cryoapplication and after double cryoapplication to the same region. A broken line indicates control value (2.0×10^{-8} g/ml). The leakage of the dye is less and is reduced below the control value more rapidly by double cryoapplication.

Double cryoapplication produced a peak fluorescein concentration in 9 of 11 eyes on the first day and in the remaining 2 eyes on the third day. The peaks ranged from 4.0×10^{-8} to 1.3×10^{-7} g/ml. The values were reduced below the control in 7 eyes on the third day (Table 2 and Fig. 1).

Twenty scattered cryoapplications produced a peak fluorescein concentration in 7 of 8 eyes on the third day with values ranging from 1.0×10^{-7} to 6.25×10^{-7} g/ml. The values fell below the control in 3 of the 8 eyes on the 35th day and in the remaining 5 on the 42nd day (Table 3 and Fig. 2).

DISCUSSION

A new objective fluorophotometer deviced by Langham and Wybar[5] was improved by Maurice.[6] It was later further developed by Waltman and Kaufman[7] into a simpler device that had fiber optics attached to the eyepiece of the photoslit lamp microscope to lead fluorescein density to the phototube. Since then fluorophotometers with built-in fiber optics have been most commonly used. Since Cunha-Vaz[8] introduced a fluorophotometer for measurement of vitreous fluorescein concentration, the vitreous fluorophotometer has been

No. \ days		1	3	5	7
No. 9		4.0	2.0	2.0	
No. 10		9.0	1.0	1.0	
No. 12		7.0	1.0	2.0	
No. 12		13.0	2.0	1.0	
No. 13		9.0	1.0	2.0	
No. 14		5.0	1.0	1.0	
No. 15		3.0	5.0	2.0	
No. 16		3.0	3.0	1.0	
No. 17	R	5.0	3.0	0	
	L	3.0	2.0	0	
No. 18		3.0	6.0	3.0	2.0
Mean ± S.D		5.8 ± 3.2	2.5 ± 1.6	1.7 ± 0.7	

Table 2. Fluorescein concentration in the mid-vitreous of double cryoapplication (g/ml × 10^{-8})

No. \ days	1	3	5	7	10	14	21	28	35	42
No. 1 R	20.0	62.5	30.0	16.4	10.9	10.0	6.6	5.6	1.9	
No. 1 L	10.0	20.0	11.3	10.0	8.2	7.3	7.3	5.6	1.9	
No. 2 R	10.0	17.5	11.0	12.7	10.0	7.3	4.6	5.6	3.7	1.3
No. 2 L	20.0	20.0	14.7	12.7	12.7	7.3	3.8	5.6	3.7	1.3
No. 3 R	13.0	30.0	36.7	32.7	18.1	18.1	11.5	6.7	4.6	0.6
No. 3 L	13.0	35.0	27.3	22.7	13.6	13.6	11.5	11.1	8.2	1.9
No. 4 R	10.0	27.5	20.0	15.4	10.9	10.9	8.5	8.9	3.7	1.3
No. 4 L	10.0	15.0	8.7	5.5	5.5	9.1	4.6	5.6	1.9	
Mean ± S.D	13.3 ± 4.4	28.4 ± 15.3	20.0 ± 10.3	16.0 ± 8.4	11.2 ± 3.8	10.5 ± 3.8	7.3 ± 3.0	6.8 ± 2.1	3.7 ± 2.1	1.3 ± 0.5

Table 3. Fluorescein concentration in the mid-vitreous of twenty scattered cryoapplications (g/ml × 10^{-8})

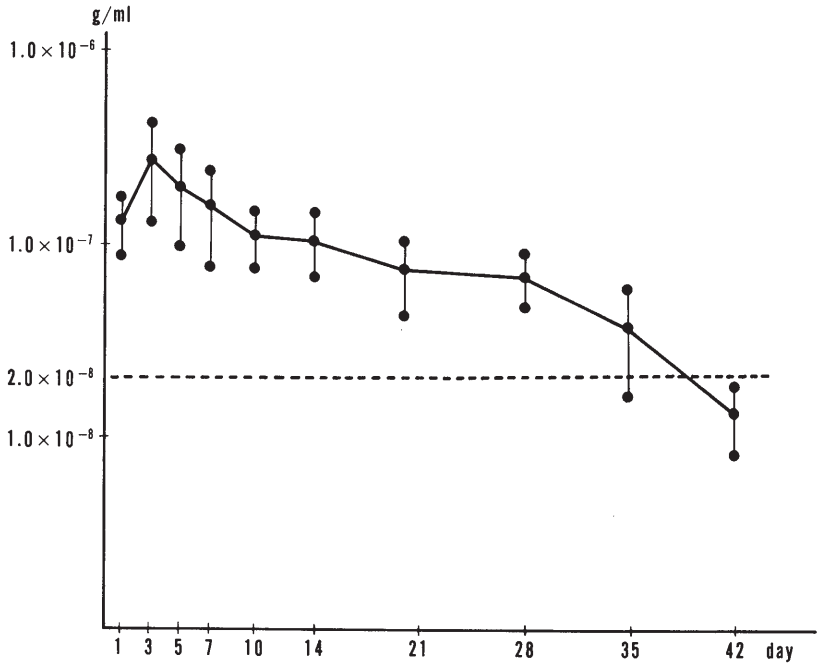

Fig. 2. The fluorescein concentration (mean ±SD) in the mid-vitreous after 20 scattered cryoapplications. Reparation required 35 to 42 days.

largely used for evaluation of various ocular diseases and has contributed to detection of the minimal degree of blood-retinal barrier damage which had escaped detection by ordinary fluorescein angiography.

It is already known that, in the normal retina, fluorescein dye injected intraveneously does not pass through the tight junction of the retinal vascular endothelium, i.e., inner blood-retinal barrier, and the tight junction of the retinal pigment epithelium, i.e., outer blood-retinal barrier, without leaking further into the retina.[9-12]

In the early (i.e., hyperglycemic) stage of diabetes, outer blood-retinal barrier damage is reported to be preceded by inner blood-retinal barrier damage, such as necrosis of the retinal pigment epithelium, uptake of the reaction product of horseradish peroxidase,[13] morphological change of the retinal pigment epithelial cells[14] and abnormal permeability of fluorescein dye through the pigment epithelium.[15] As vitreous fluorophotometry permits detection of the elevated vitreous fluorescein concentration in patients with early diabetes in whom fluoroangiography fails to detect the leakage of the dye from the retinal vessels,[16] it is also regarded as being helpful in the diagnosis of outer blood-retinal barrier damage.

The present experiment was undertaken to evaluate the degree of outer barrier injury resulting from cryoretinopexy. The course of post operative reparation was also evaluated, since in surgical management of retinal detachment, especially in highly detached cases, it may take a long time from choroidal cryocautery until the growth of white cryospots on the retina, and repeated cautery to the same region twice or more, or profuse cauteries may be applied when giant or a number of tears are present.

In rabbit eyes treated with ordinary cryoapplication and double cryoapplication, fluorescein leakage was reduced below the control 7-10 days and 3-7 days, respectively, after

the operation. The histopathological pattern of the lesion a week after ordinary cryoapplication demonstrated that the retinal pigment epithelium that had once been destroyed was repaired and covered, though insufficiently, the Bruch's membrane. At 2 weeks after cryoapplication, the Bruch's membrane was almost entirely covered by the regenerated pigment epithelium (Fig. 3). In contrast, by double cryoapplication, the retina was severely damaged with the pigment epithelium only covereing the Bruch's membrane in an irregular, stepping-stone-like manner (Fig. 4).

Concerning retinal changes by cryosurgery, Lincoff[17-19] reported that a thin pigment epithelium which covered the Bruch's membrane within a week, was regenerated from the periphery of the lesion, and the regenerated pigment epithelium was connected with Müller cells by desmosomes on the 14th day. Though a reconstruction of the tight junction at one week after cryoapplication was not mentioned, the time of the covering of the Bruch's membrane by the pigment epithelium well agrees with the results of the present experiment. On reparation of the barrier after cryocautery, Smith *et al*[1] described that in their experiment of cryoretinopexy in monkeys using horseradish peroxidase as a tracer, the tracer reached the outer layer of the junctional complex at 2-4 weeks after the operation but not further interiorly. Their findings are highly consistent with the present results that demonstrated the reduction of the fluorescein concentration below the control value 10 days after the opertion.

The present study produced seemingly contradictory findings in that the barrier damage from double cryoapplication was repaired more rapidly than damage from ordinary cryoapplication. Histologically as well as ophthalmoscopically, damage of the retina was more severe by double cryoapplication than by ordinary application.[20] The histopathological findings obtained in this study also showed only a stepping-stone-like covering of the pigment epithelial cells over the Bruch's membrane as a result of double cryoapplication (Fig. 4). This finding is similar to that of migration of the pigment epithelial cells after cryopexy,[19] and it is considered that the repair of the pigment epithelium has not been accomplished. Nevertheless, the fact that fluorescein concentration had been reduced below the control might suggest the possibility that the reduction of leaking fluorescein correlated to the reduced blood flow through the injured choriocapillary in such intense cryopexy as double cryoapplication. Therefore, in spite of the reduction of leaking fluorescein, it is conjectured that the repair of the blood-retinal barrier damage from double cryoapplication will take more time than repair of damage from ordinary cryoapplication.

The larger leakage of dye in the 20 scattered cryoapplications was a natural outcome, and leakage was prolonged to 35-42 days. Prolongation of the leakage may be attributed to the fact that despite a very small amount of leakage from individual leaking spots, the total amount of leakage from 20 scattered spots exceeded that from 8 spots. Furthermore, during 20 scattered cryoapplications in rabbits eyes, even with maximum care to avoid overlapping of cryospots, the cryospots were postoperatively found to be partially linked together. If regeneration of the pigment epithelium is achived by movement of surrounding intact pigment epithelium, the delayed repair of the pigment epithelium in the central area of the aggregated lesions may be considered as entirely natural. From the results of Zweig[21] showing that by panphotocoagulation with argon laser in pigmented rabbit eyes, the damage returned almost to the control value in 30 days by mild photocoagulation but failed to recover even 90 days later by severe photocoagulation, it seems that the number and intensity of the applications are correlated with the destruction of the barrier.

Adding to such retinal problems as thinning and break formation arising from excessive cryoapplication, the above may indicate that in terms of the outer barrier damage associated

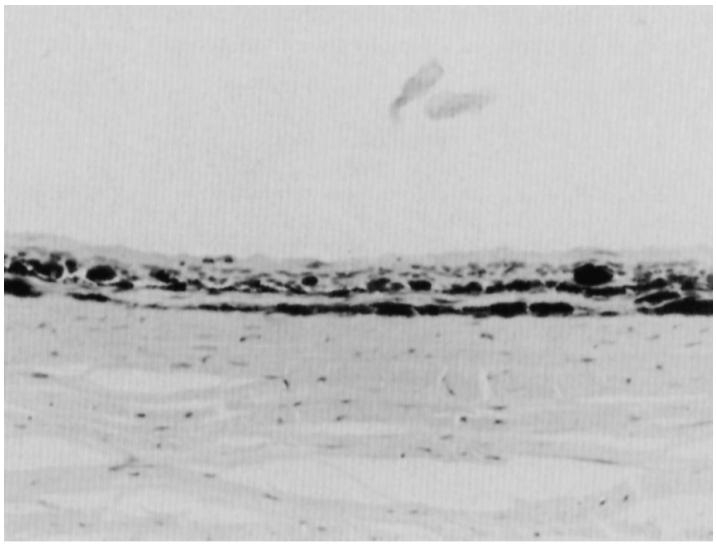

Fig. 3. Reparation with the regenerated retinal pigment epithelium is almost completed 2 weeks after ordinary cryoapplication.

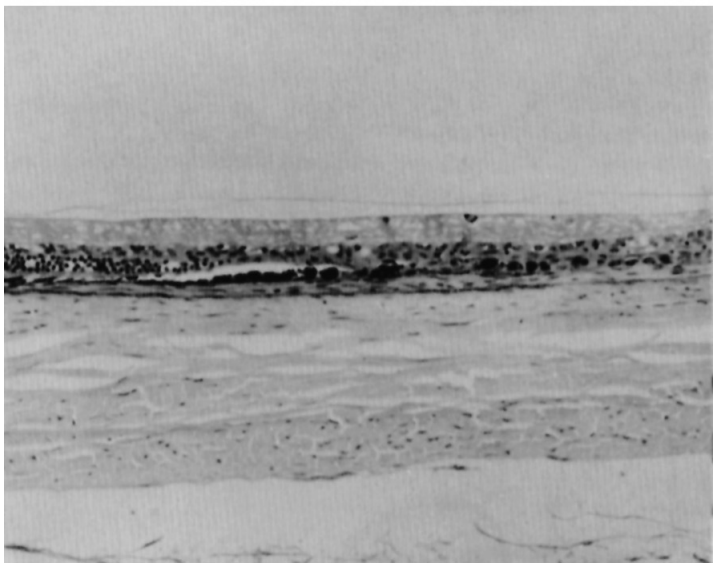

Fig. 4. Despite cessation of the leakage, the pigment epithelium is repaired only sporadically 2 weeks after double cryoapplication.

with the pigment epithelium, utmost care should be taken to avoid double cryoapplication to the same region. And it should be kept in mind that the more extensive cryoapplications at one time may result in the more adverse effects on the blood-retinal barrier.

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