The permeability of the posterior blood ocular barrier after xenon photocoagulation: a study using fluorescein labelled dextrans

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SUMMARY Xenon photocoagulation burns in the rabbit fundus were studied angiographically with fluorescein labelled dextrans of molecular weights in the range 3000 to 150 000. Recent photocoagulation burns showed dye leakage to all molecular weights used. Angiograms 2 days after burns had been produced showed leakage of dextrans of molecular weights up to and including 70 000 but no leakage of dextran of 150 000 molecular weight. At 7 days after photocoagulation healed burns remained permeable to dextrans of molecular weight 3000 or 20 000, but in the majority of eyes such burns did not leak dextrans of over 40 000 molecular weight. The clinical significance of the selective nature of photocoagulative damage to the posterior blood ocular barrier is discussed.

It is well recognised clinically that recent photocoagulation burns leak fluorescein on angiography. In the rabbit experimental burns leak fluorescein for up to 3 days but not subsequently,¹ though electronmicroscopy shows that healed burns remain abnormally permeable to small tracers such as horse radish peroxidase.^{2,3} As it is possible that molecular size is important in relation to the permeability of the posterior blood ocular barrier after photocoagulation, we studied the permeability of photocoagulation burns to fluorescein labelled dextrans of various known molecular sizes. A preliminary report of this work has already been published,⁴ and the study has now been amplified by further work reported here.

Materials and methods

Fluorescein isothiocyanate (FITC) labelled dextrans (Pharmacia Fine Chemicals) were used throughout this study in the following molecular weights: 3000, 20 000, 40 000, 70 000, and 150 000. A molecular weight of 70 000 corresponds approximately in molecular size to plasma albumin and that of 150 000 to plasma globulin.

FITC dextrans are a homologous series of glucose

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polymers. They are covalently labelled with fluorescein by condensation with fluorescein isothiocyanate. Free fluorescein is removed by ethanol precipitation and molecular sieve chromatography.⁵ When incorporated in the dextran molecule the fluorescein remains firmly conjugated, but the actual concentration of fluorescein in the dextran is low, and correspondingly high doses had to be used for angiography. In the present experiments 1 g of FITC dextran was used in each angiographic study. With the lower molecular weights this amount of dextran could be dissolved in 2 ml of distilled water, but with the higher weight dextrans the solutions were very viscous and up to 6 ml of distilled water was required to dissolve the dextran.

The animals used in this study were Dutch rabbits of 1.5 to 2.5 kg body weight. For photocoagulation the animals were anaesthetised with 0.5 to 1.0 ml of intravenous pentobarbitone sodium, 60 mg per ml, and for the angiographic studies, which were terminal, the animals were anaesthetised with 5 to 10 ml of intravenous ethyl carbamate (urethane) 40 mg per ml. Pupillary dilatation was achieved with topically applied guttae cyclopentolate hydrochloride 1% *BP* and guttae phenylephrine hydrochloride 10% *BP*.

In each experimental animal a series of photocoagulation lesions were produced, so that in the majority of animals there were identifiable burns of 7 days', 2 days', and 1 hour's standing (Fig. 1). A Zeiss

Fig. 1 Fundus photograph showing photocoagulation burns of 7 days' (A), 2 days' (B), and 1 hour's duration (C).

Jena Liko 500 xenon arc photocoagulator was used to produce 5° burns of an intensity which would be considered within the therapeutic range.

Before angiography the animal was anaesthetised and a femoral artery was surgically exposed and cannulated. A colour photograph of the fundus was

taken and this was followed by angiography using FITC dextran of one of the molecular weights available. 1 g FITC dextran dissolved in 2–6 ml distilled water was injected into the femoral artery in as short an interval as possible, varying from 20 seconds with the lower molecular weight dextrans to 60 seconds with the more viscous higher molecular weight dextrans, and angiograms were taken with a Nikon 45° camera equipped with interference exciter and barrier filters. Ilford SP 4 film was used and was conventionally processed. The first frame was exposed simultaneously with the start of the injection of dextran. Further frames were recorded at 2-second intervals for 5 minutes and thereafter at 5-minute intervals for 15 minutes, 30 minutes, or in some instances 60 minutes. The animal was then killed with an overdose of anaesthetic and the eyes were enucleated (in some cases after the injection of electronmicroscopy tracers) and fixed for histological and electronmicroscopical studies.

Individual photocoagulation burns on the angiograms were classified according to the following definitions:

(1) Intact blood ocular barrier (Fig. 2). Lesions showing no hyperfluorescence during dye transit or showing discrete fluorescence matching the background choroidal fluorescence in intensity and duration, that is, 'show through' (A).

(2) Possible abnormality in the blood ocular barrier (Fig. 2). Lesions which became hyperfluorescent during the dye transit with an intensity

2a

2b

Fig. 2 FITC dextran angiography to illustrate (A) intact blood ocular barrier; (B) 'Staining'; (C) 'Leakage'. 2a: 137 s. 2b: 30 min.

which was greater than the background choroidal fluorescence but which remained discrete and localised within the area of the photocoagulation lesion, that is, 'staining' (B).

(3) Definite breakdown in blood ocular barrier (Fig. 2). Lesions which became progressively more hyperfluorescent during the dye transit, with an intensity greater than the background choroidal fluorescence and with a fluorescence which spread beyond the confines of the burn, that is, 'leakage' (C).

It should be noted that the rate of excretion of FITC dextrans by the kidney depends on the molecular weight, the higher molecular weight dextrans remaining in circulation for longer than the lower. This affects the interpretation of the angiograms, choroidal showthrough, for example, being a longer lasting phenomenon in relation to the higher weight dextrans than to the lower.

Results

One hundred and fifty-eight photocoagulation burns were assessed. The numbers of burns, molecular weights of FITC dextran used, and the time interval between the coagulation and angiography are shown in Table 1.

The behaviour of burns on angiography varied with the time interval between the coagulation and angiography and with the molecular weight of FITC dextran used. The results will be presented in accordance with the time intervals used.

Photocoagulation burns 1 hour after production. All burns at this time showed a breakdown of the blood ocular barrier with copious leakage of all molecular weights of FITC dextran used, confirming that recent photocoagulation burns are permeable to both small and large molecules (Fig. 3).

Photocoagulation burns 2 days after production. Healing photocoagulation burns of this age showed leakage of FITC dextrans of all molecular weights up to and including 40 000. With the highest molecular weight used (150 000) half of the burns showed stain-

Numbers % burns leaking or staining

Fig. 3 Diagram to illustrate, at different times, change in the permeability of the blood ocular barrier after photocoagulation, as tested with FITC dextrans of various molecular weights.

ing, while the rest showed no abnormal fluorescence or at the most showthrough only (Fig. 3). The majority of those treated with 70 000 MW showed either leakage or staining.

Photocoagulation burns 7 days after production. At this stage the behaviour of burns varied greatly with the molecular weight of the FITC dextrans used.

3000 Molecular weight dextran. Of 25 burns studied 14 (56%) showed definite leakage and a further 11 (44%) showed staining. In no case was the blood ocular barrier considered intact. In the majority of instances the leakage or staining became manifest 15 minutes after dye injection, but in a few instances it was earlier or later (Table 2) (Fig. 4).

20000 Molecular weight dextran. Of 10 burns studied 4 (40%) showed leakage and a further 3 (30%) staining. Three burns (30%) were considered to show no abnormality in the blood ocular barrier. Again most of the burns showing hyperfluorescence developed this 15 minutes after dye injection.

40000 Molecular weight dextran. There were 11 burns in this group and none showed frank leakage. In 2 (18%) late staining developed 45 minutes after

Table 1 Number of animals and number and duration of photocoagulation lesions studied angiographically with dextrans of various molecular weights

Dextran molecular weight	Number and duration of PC lesions			Number of animals
	1 hour	2 days	7 days	
3000	4	21	25	7
20000	2	7	10	2
40000	7	10	11	3
70000	6	16	15	5
150000	2	9	13	4
Totals	21	63	74	21

PC=photocoagulation.

Fig. 4 Angiogram using 3000 MW FITC dextran to show 'leakage' of burns up to and including those of 7 days' duration. (A) 1 hour; (B) 2 days; (C) 7 days. 4a: 56 s. 4b: 187 s. 4c: 259 s.

Table 2 Time of appearance of leakage and staining after dye injection using 3000 molecular weight dextran

3000 MW dextran 25 burns				
Result	Delay after dye injection			
	5 min	15 min	30 min	
Leakage	3	9	2	
Staining	2	6	3	
Totals	5	15	5	

dye injection. Nine of the 11 burns (82%) showed neither leakage nor staining.

70 000 Molecular weight dextran. Of 14 burns examined 2 (14%) developed late leakage at 45 minutes after dye injection and a further 2 (14%) showed staining. In 10 burns (72%) there was neither leakage nor staining.

150 000 Molecular weight dextran. None of the 14 burns examined showed either leakage or staining (Fig. 5).

Discussion

In holangiotic retinae the posterior blood ocular barrier is known to reside in the tight intercellular junctions between retinal capillary endothelial cells⁶ and between cells of the retinal pigment epithelium (RPE).⁷ In the anangiotic retina of the rabbit the posterior blood ocular barrier is presumed to be formed exclusively by the retinal pigment epithelium with its apical tight intercellular junctions. This barrier prevents the passage across the RPE of intravenously or intravitreally injected electron dense tracers such as horse radish peroxidase,^{2,3} which has a molecular weight of around 40 000.

Shortly after photocoagulation the RPE barrier is completely disrupted and can be shown to be permeable not only to small electron microscopy tracers

such as horse radish peroxidase but also to large particles such as colloidal carbon.³ Within a week photocoagulation burns cease to leak fluorescein on conventional angiography,¹ though electron microscopy may show that the healed burns are still permeable to horse radish peroxidase.^{2,3} In conventional fluorescein angiography most of the circulating fluorescein is bound to plasma albumin, with a molecular weight of around 70 000, and the discrepancy between the apparent permeability of healed photocoagulation burns to horse radish peroxidase (MW 40 000) and the lack of fluorescein leakage on conventional angiography may be an expression of the

Fig. 5 Angiogram using 150 000 FITC dextran. Both 2-day burns (A) and 7-day burns (B) demonstrate an intact blood ocular barrier.

difference in molecular weight between plasma albumin and horse radish peroxidase.

The results presented in this paper indicate that fresh photocoagulation burns in the rabbit are freely permeable to dextrans of molecular weights up to 150 000. After 2 days the permeability of burns is reduced in respect of the higher molecular weights, and at this stage with 150 000 MW dextran half the burns showed neither leakage nor staining, while most burns at this stage tested with lower molecular weight dextrans in the range 3000 up to 40 000 showed leakage. At 7 days it has been shown by electron microscopy that numerous new intercellular junctions have formed within the healed burn,¹³ though such burns remain permeable to horse radish peroxidase as shown by electron microscopy. At this stage leakage of dextrans of 3000 and 20 000 molecular weight was commonly seen, while for dextrans of higher molecular weight the incidence of leakage was greatly reduced, no leakage or staining being seen with dextran of molecular weight 150 000. For dextrans of 40 000 and 70 000 together only 23% showed leakage or staining at a week. The changing permeability to dextrans of differing molecular weights during the healing of photocoagulation burns is shown graphically in Fig. 3 and illustrates the fact that photocoagulation burns may selectively damage the posterior blood ocular barrier, so that even in healed burns permeability to small molecules may be abnormally increased, while the barrier remains impermeable to large molecules such as plasma protein.

The findings are of interest in relation to the therapeutic use of photocoagulation in diabetic retinopathy. In proliferative retinopathy, if a diffusible vasoformative factor is elaborated by ischaemic retina, it is theoretically possible that multiple photocoagulation burns might modify the blood ocular barrier in such a way as to reduce the level of vasoformative factor in the eye. Previously it has been suggested¹ that the clearance of subretinal fluid from the eye following retinal detachment procedures and after nonspecific photocoagulation to the

paramacular area in cases of macular oedema might also be the result of an increased movement of fluid across an abnormally permeable RPE cell layer, and the present findings would not conflict with this view. To be effective such a movement would require the presence of a hydrostatic pressure difference between the vitreous or retina and the choroid, and as yet no such pressure difference has been detected.⁸ Even a very slight pressure difference, however, could have a significant effect on flow,⁹ as indeed could the onchotic pressure in the choroid acting across a blood ocular barrier made selectively permeable to small molecules while retaining its impermeability to plasma protein.

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