Permeability of the blood-ocular barrier in adolescent and adult diabetic patients

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Abstract

The permeability of the blood-ocular barrier was examined by fluorophotometry in adolescent and adult diabetic patients before the onset of retinopathy. The adolescent group consisted of 52 eyes of 52 insulin dependent diabetic patients aged 11 to 19 years and a control group of 10 eyes of 10 normal adolescents. The adult group consisted of 74 eyes of 74 non-insulin dependent diabetics and a control group of 30 eyes of 30 normal adults. The increase in lens autofluorescence in the adolescent diabetic patients compared with the controls was striking and showed a significant positive correlation ($r=0.79$, $p<0.0001$) with the duration of diabetes. Anterior chamber (AQ) values, an index of the permeability of the blood-aqueous barrier (BAB), increased in the adolescent diabetic patients compared with the controls and showed a significant positive correlation with glycosylated haemoglobin levels. No significant differences from the controls were observed regarding the permeability of the blood-retinal barrier (BRB). In the adult group there was no significant difference in either the permeability of the BRB or the AQ values between the diabetic and the control groups. Our results suggest that adolescent diabetic patients differ from adults in that BAB permeability is increased before the onset of retinopathy, suggesting that this is the cause of the striking increase in lens autofluorescence.

(Br J Ophthalmol 1993; 77: 158–161)

Adolescent and adult diabetic patients seem to differ somewhat in clinical character: the adolescents are mostly insulin-dependent diabetes mellitus (IDDM) cases, and their blood sugar tends to be difficult to control during puberty. In addition, the longer duration of the diabetes can be expected to result in major eye problems for adolescent diabetics later in life. However, the difference between adolescent and adult diabetics in terms of retinopathy is still unclear. Using vitreous fluorophotometry, some authors[1][2] reported increased permeability of the blood-retinal barrier (BRB) before the onset of a detectable retinal vascular abnormality in at least some patients with diabetes. Bursell et al[3] evaluated the integrity of the BRB by vitreous fluorophotometry in younger (20–40 years) and older (50–70 years) adult diabetic patients with different degrees of retinopathy in age-matched normal volunteers. They found a greater BRB permeability in the younger than in the older diabetic group, compared with the respective controls. No further investigations have been reported.

In the present study, we evaluated the permeability of the blood-ocular barrier (BOB) before the onset of retinopathy in both adolescent and adult diabetics and found different patterns of impairment of the permeability in the two groups. These findings may help to elucidate the mechanism of visual impairment in adolescent diabetes.

Subjects and methods

SUBJECTS

Legal requirements governing informed consent were fulfilled for all subjects. Selection of the right or left eye was random. The adolescent group comprised 52 eyes of 52 Japanese IDDM patients (25 male, 27 female) aged 11 to 19 years (mean, 14.9 years) (Table 1). Ophthalmoscopy or fluorescein angiography did not reveal any diabetic retinopathy in this group. Ten eyes of 10 age-matched Japanese adolescents (five male, five female) in normal health served as controls. The adult group comprised 74 eyes of 74 Japanese, non-insulin-dependent diabetes mellitus (NIDDM) patients (39 male, 35 female) aged 41 to 59 years (mean, 49.8 years), and ophthalmoscopy or fluorescein and angiography did not reveal any retinopathy. Thirty eyes of 30 age-matched Japanese adults (16 male, 14 female) in normal health served as controls. Eyes with refraction errors of ±1.0 D or higher, which were considered likely to affect the fluorophotometry values,[4] and eyes with posterior vitreous detachment[5] were excluded. The mean duration of diabetes was 5.2 years for the adolescent group and 6.9 years for the adult group; the difference was not significant ($p>0.05$ by Student's t test).

FLUOROPHOTOMETRY

Both pupils of all subjects were fully dilated with 0.5% phenylephrine hydrochloride and 0.5% tropicamide. Because diabetic patients sometimes tend to have smaller pupils following dilatation than do non-diabetic controls, which could affect the vitreous fluorophotometry results, we tried to achieve maximum pupil

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Table 1 Characteristics of subjects

	Adolescent group	Adult group
Diabetics		
Number	52	74
Duration (years) (SD)	5.2 (3.7)	6.9 (5.3)
Age (years) (SD)	14.9 (2.3)*	49.8 (5.2)*
Controls		
Number	10	30
Age (years) (SD)	13.9 (2.2)	49.5 (6.0)

*Not significantly different from control group.

dilatation (7·5–8·0 mm) and excluded patients with poor pupil dilatation from the study.

The fluorophotometer used has been described previously.[6-10] Briefly, we employed a modified Haag-Streit model 360 slit-lamp to record equivalent fluorescein concentration profiles in the ocular media. The angle between illumination and measuring light paths was 14 degrees, and the beam dimensions at the focal plane in the air were 3·0 mm×150 μm and 2·8 mm×150 μm, respectively. The axial resolution of the instrument is 1·85 mm, and sample volume is 0·38 ml.[6] The meter readings of this system were related linearly to the fluorescein concentrations in a range from $7·0×10^{-11}$ to $8·0×10^{-7}$ g/ml. The average reproducibility of the measurements was determined previously[6] from the lens autofluorescence and the vitreous value 3 mm from the retina at the preinjection baseline recording as well as the calculated inward permeability of the BRB (P_{in}) following the injection of 10% sodium fluorescein in 10 eyes of 10 normal subjects. Two measurements were made on each subject at different times under the same experimental conditions. The average reproducibility was 4·3% for lens autofluorescence, 8·5% for vitreous values and 10·0% for P_{in} values.

After topical anaesthesia with 0·4% benoxinate hydrochloride for each measurement, a low vacuum contact lens was placed on the cornea using methylcellulose solution. All measurements along the optical axis were taken before (baseline) and 60 minutes after antecubital vein injection of 10% sodium fluorescein (Fluorescite, Alcon, Fort Worth, TX, USA) at a dose of 7 mg/kg of body weight.

To determine the concentration of free, protein unbound fluorescein (PUF) in plasma, blood was taken from the antecubital vein on the side opposite the dye injection at 10 and 65 minutes after fluorescein injection by a method described previously.[11] Briefly, plasma was separated from 2 ml of blood by centrifugation at 1500 g for 15 minutes, and then centrifuged in an ultrafiltration membrane cone (MPS-1, Amicon, Danvers, MA, USA) at 2000 g for approximately 15 minutes. The fluorescence of the ultrafiltrate then was measured with a fluorophotometer.

Since the autofluorescence of the crystalline lens can be recorded as the large signal that has one peak with a slit-lamp fluorophotometer like ours,[3 12] the lens autofluorescence was obtained from the centre of the lens peak.

To determine the permeability of the BRB using our computer simulation technique,[7 13] we adopted the values from 14 dispersed sampling points. Serial vitreous fluorophotometry measurements were used to derive these points. For example, to determine the anterior vitreous value, seven points were used with a 0·5 mm interval starting at a point 2·5 mm posterior from the centre of the crystalline lens. To determine the posterior vitreous value, seven points were used with a 0·5 mm interval located between 3·0 and 6·0 mm anterior to the retinal surface. These vitreous fluorophotometry readings were corrected for the baseline value to calculate the fluorescein concentrations.

Based on this eye model, dye kinetics in the vitreous cavity were simulated, and the P_{in} and the diffusion coefficient in the posterior vitreous $(D-p)$ were estimated for each eye.[7 13]

Because it is difficult to derive a permeability coefficient of the blood-aqueous barrier (BAB) with our present simulation method, anterior chamber (AQ) values were used as indices in estimating the permeability of the BAB. To obtain AQ values, baseline fluorescence was subtracted from the 60-minute postinjection value at the centre of the anterior chamber, and then these figures were divided by the dynamic changes in PUF concentration:

$$AQ\ values = \frac{60\text{-min AQ values} - \text{baseline AQ values}}{\int_0^{60} Cp\,(t)\,dt}$$

Table 4   Permeability of the blood-aqueous barrier (AQ value, $×10^{-2}\,h^{-1}$)

	Adolescent group	Adult group
Diabetics (SD)	2·0 (0·8)* (n=52)	2·5 (1·1)† (n=74)
Controls (SD)	0·9 (0·3) (n=10)	2·3 (1·1) (n=30)

AQ=anterior chamber.
*Significantly different from control group, p<0·0001.
†Not significantly different from control group.

Table 2   Lens autofluorescence ($×10^{-7}$ g/ml fluorescein equivalent)

	Adolescent group	Adult group
Diabetics (SD)	0·7 (0·4)* (n=52)	2·5 (0·8)* (n=74)
Controls (SD)	0·4 (0·05) (n=10)	1·9 (0·4) (n=30)

*Significantly different from control group, p<0·01

Table 3   Permeability of the blood-retinal barrier

	Adolescent group		Adult group	
	P_{in} ($×10^{-6}$ cm/min)	$D-p$ ($×10^{-4}$ cm²/min)	P_{in} ($×10^{-6}$ cm/min)	$D-p$ ($×10^{-4}$ cm²/min)
Diabetics (SD)	7·9 (3·9)*	9·1 (4·2)*	8·6 (3·6)*	17·9 (10·8)*
	(n=52)		(n=74)	
Controls (SD)	6·4 (2·5)	9·1 (4·7)	9·1 (3·1)	18·4 (14·7)
	(n=10)		(n=30)	

P_{in}=inward permeability of the blood-retinal barrier; $D-p$=diffusion coefficient in posterior vitreous.
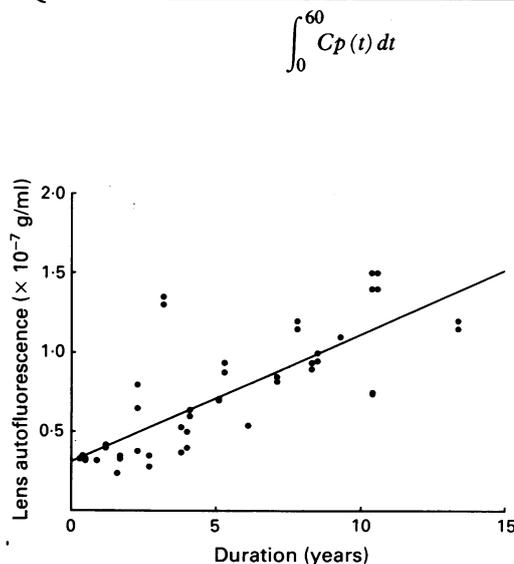*Not significantly different from control group.

Figure 1   Relationship between duration of diabetes and lens autofluorescence, expressed in equivalent fluorescein concentrations, in the adolescent diabetics. A statistically significant correlation is seen, r=0·79 (p<0·0001).

Table 5 Correlations involving systemic factors

	Adolescent diabetics	Adult diabetics
Duration of diabetes vs lens autofluorescence	r=0·79 (p<0·0001)	r=0·04 (NS)
HbA$_{1c}$ vs P_{in}	r=−0·10 (NS)	r=0·32 (p<0·01)
HbA$_{1c}$ vs AQ value	r=0·32 (p=0·03)	r=−0·12 (NS)

HbA$_{1c}$=glycosylated haemoglobin level in blood; P_{in}=inward permeability of the blood-retinal barrier; AQ=anterior chamber value; NS=not statistically significant.

Here, $Cp(t)$ is the PUF time function.[7][11]

DATA ANALYSIS

The data were analysed using standard statistical methods. Student's *t* test was used to compare groups. Differences were considered significant when the probability value indicated a chance of random occurrence of less than 5%.

SYSTEMIC FACTORS IN DIABETICS

Systemic factors in diabetics considered in this study were the duration of diabetes and the status of blood sugar control, as indicated by glycosylated haemoglobin (HbA$_{1c}$) and fasting blood sugar levels. For the adolescent group in particular, the concentration of microalbumin in the urine was measured by radioimmunoassay as an index of renal impairment, and motor nerve conduction velocity (MCV) in the right median nerve was measured as an index of peripheral nerve impairment, using the 7S12 Signal Processor (Sanei, Tokyo, Japan).

Results

FLUOROPHOTOMETRY

Table 2 compares lens autofluorescence in the diabetic and control groups obtained from baseline fluorophotometric measurements. For both the adolescent and adult groups, diabetics scored significantly higher values than did the controls (p<0·01). The average lens autofluorescence of adolescent diabetics was 1·8 times higher than the control group and of adult diabetics 1·3 times higher than the control group.

Table 3 compares P_{in} and $D-p$ values in the diabetic and control groups. In both the adolescent and adult subjects, there was no significant difference between the diabetic and control groups in either P_{in} or $D-p$ values.

Conversely, with regard to AQ values (Table 4), which are indicators of the permeability of the BAB, significantly higher values were seen in the adolescent diabetics compared with the adolescent control group (p<0·0001); virtually no difference was seen between the two adult groups.

RELATIONSHIP TO SYSTEMIC FACTORS

Figure 1 indicates the significant positive correlation between the duration of diabetes and lens autofluorescence in the adolescent diabetics (r=0·79, p<0·0001). Table 5 shows the correlation between systemic factors and lens auto-

fluorescence, P_{in} and AQ value. No ocular factor correlated significantly with either the MCV values or the microalbumin concentration in the urine.

Discussion

Before the onset of retinopathy in adult diabetics, lens autofluorescence values are higher than in age-matched normal controls.[3][14][15] Although there has been no study specifically devoted to adolescent diabetic patients, Sparrow *et al*[16] have shown increased fluorescence of the lens in diabetic patients under the age of 20. Our present study demonstrates that adolescent diabetics, like adults, display high lens autofluorescence values before the onset of retinopathy. Further, our results indicate that lens autofluorescence is 1·8 times higher than normals among adolescent diabetics, but only 1·3 times higher than normals among adult diabetics. In addition, we found that high lens autofluorescence values in adolescent diabetics have a significant positive correlation (r=0·79, p<0·0001) with the duration of the illness.

Bleeker *et al*[15] showed the lens autofluorescence values in adult diabetics are influenced by both age and the duration of diabetes. In our study, the average age of the adolescent diabetics was 14·9 (SD 2·3) years, a homogeneous group with respect to age; thus, the influence of age on lens autofluorescence can be virtually disregarded in our adolescent diabetics. Based on our results and those of Bleeker *et al*[15] lens autofluorescence in adolescent diabetics is higher than in normals, depending on the duration of diabetes, at least at the stage before the onset of retinopathy.

It has been suggested that the increased lens autofluorescence in diabetics results from changes similar to those observed in normally aging or cataractous human lenses.[17] In the aging lens, increases in lenticular scatter have been related to increased concentrations of high molecular weight protein aggregates in the lens nucleus.[18][19] The exact nature of the lens fluoropigment is unknown, but it is thought to be related to photo-oxidation products of tryptophan.[20] The lens autofluorescence measured here results from the combined effect of the lens fluoropigment and the scattering within the lens of both the excitation beam and the emitted autofluorescence. Whatever the case, increased lens autofluorescence in diabetics may be closely related to the formation of sugar cataracts.

The second significant result of our study is that adolescent diabetics differ from adult diabetics in that significantly higher AQ values are seen before the onset of retinopathy and also before any increase in the permeability of the BRB. Our results are consistent with the findings of previous research. Ishibashi *et al*[21] reported a pattern of increased permeability in the iridial BAB in streptozotocin-diabetic rats, as observed under the optical and electron microscopes, using horseradish peroxidase as a tracer. Klein *et al*[22] performed fluorescein angiography of iris and retina in 100 diabetics and 59 normal subjects and found that the first signs of diabetic vascular damage can be seen earlier in the iris than in the retina. Therefore, a comparison of

our results with earlier reports strongly suggests that the permeability of the BAB increases earlier than the permeability of the BRB in adolescent diabetes. Moreover, the significant positive correlation between AQ values and HbA$_{1c}$ levels points to the possibility that the permeability of the BAB may be influenced by blood sugar control.

We consider that the two striking differences between adolescent and adult diabetics clarified from our study – increases in both lens autofluorescence and the permeability of the BAB in the adolescents – are not discrete but fundamentally the same – that is, the composition of the aqueous fluid changes in accordance with functional changes such as increased permeability of the BAB, and it is surmised that as the result of this, an increase in autofluorescence – that is, a change within the lens, occurs as a secondary change.

It is also surmised that blood sugar control affects the permeability of the BAB in adolescent diabetics. In teenage IDDM patients it is relatively common for blood sugar levels to change markedly in the course of a day, in contrast to adult diabetics, even in cases for which blood sugar control is regarded as comparatively good. Under normal conditions, the interendothelial junctions of the retinal vessels are known to be extremely tight, but the interendothelial junctions of the iris vessels are 10 times more permeable. They are known as 'leaky' junctions.[23] In this context, we have speculated as to whether changes in blood sugar levels in the course of a day in adolescents first impair the BAB, which is more fragile than the tighter BRB.

The present study confirmed our clinical impression that adolescent and adult diabetics are different, particularly with regard to impairment of the permeability of the BOB, and in particular the BAB. It is therefore essential to consider these differences when managing adolescent diabetics in order to minimise ocular complications.

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