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Effect of calcium dobesilate on the blood-retinal barrier in early diabetic retinopathy

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Summary

The effect of calcium dobesilate on the alteration of the blood-retinal barrier was studied in 41 adult-onset, non-insulin dependent diabetic patients with minimal or no retinopathy, randomly assigned to receive either oral calcium dobesilate (1000 mg twice daily) or a placebo for 12 months. The posterior vitreous value and the penetration ratio, determined by vitreous fluorophotometry, reflected stabilisation of blood-retinal barrier permeability in the calcium dobesilate patients and deterioration of blood retinal barrier in those given placebo. During the relatively short period of the study, one year, no significant change in microaneurysm and capillary closure gradings was observed. No side effects were associated with calcium dobesilate.

Introduction

An alteration of the blood-retinal barrier is among the first retinal changes in diabetes mellitus [2, 3]. Therefore, research on the earlier stages of diabetic retinopathy when visual acuity is still 20/20 should include fluorescein angiography and vitreous fluorophotometry.

Administration of sulindac or sorbinil for periods of six months has been shown to stabilise the blood-retinal barrier in early diabetic retinopathy [5, 6], but serious adverse reactions have been reported in association with these compounds although our clinical trials did not reveal major side effects. Freedom from side effects is especially necessary in drugs for early diabetic retinopathy, because such drugs are liable to be used for a long time, the patients are symptom-free and at last the disease is unpredictable.

Calcium dobesilate (calcium 2,5–dihydroxybenzenesulfonate) (Doxium), which has been prescribed in Europe for diabetic retinopathy for many years, has emerged from clinical studies [9, 13, 16] as conferring possible benefit but not giving rise to side effects.

We report here a double-blind study designed to observe the effect of calcium dobesilate on the initial stages of diabetic retinopathy, with special attention to the changes of the blood-retinal barrier as measured by vitreous fluorophotometry.

Subjects and methods

The study was a randomised, double-blind, clinical trial of calcium dobesilate versus placebo, conducted in adult-onset diabetic patients with minimal or no retinopathy and based on retinopathy grading and measurements of the permeability of the blood-retinal barrier.

Study design and eligibility

Forty-seven patients with non-insulin dependent diabetes mellitus (NIDDM) were enrolled from our out-patients clinic. Details of the trial were explained and consent was obtained. A random allocation method assigned 23 patients to receive calcium dobesilate and 24 patients to receive a placebo. The key to the randomisation codes was kept by the local pharmacist and was unknown to the examiners until the trial was completed.

Excluded from the trial were patients with the following conditions: more than level 3 of the modified Airlie House classification of diabetic retinopathy [7]; vitreous detachment; cloudy ocular media or a refraction correction greater than 5 diopters; vascular, neurological or dermatological complications of diabetes; heart disease; hypertension (diastolic blood pressure greater than 90 mmHg or systolic blood pressure greater than 160 mmHg); stroke; hyperthyroidism; active peptic ulcer; gastrointestinal haemorrhage; hepatic, renal or haematopoietic disorders; or malignant disease.

Calcium dobesilate was given in a dosage of 2 × 500 mg capsules twice daily for 12 months. Compliance tested by counting returned capsules at each visit was considered good. Moreover, calcium dobesilate measured in the plasma by high-pressure liquid chromatography (HPLC) was identified in those patients taking the drug.

All the patients had the following investigations: visual acuity, slit-lamp examination and ophthalmoscopy at 0, 3, 6, 9 and 12 months; fundus photography, fluorescein angiography and vitreous fluorophotometry at 0, 6 and 12 months; and a diabetological examination and laboratory tests (including urinalysis, complete blood cell count, haemoglobin A1 determination, blood glucose and blood creatinine estimation) every 3 months.

Four of the original 47 patients, two from each treatment group, were excluded because they did not attend the subsequent examinations. Out of the 43 patients who completed the 12-month trial, one from each group, was also excluded from data analysis because they were considered outliers due to exceptionally high fluorophotometric values (patient #26, on active drug: PR – 20.46/38.22 × 10^{-6}.min^{-1} patient #23, on placebo: PR/19.70/37.21 × 10^{-}.min^{-1} at start/end of the trial). Data analysis was therefore finally done in 41 patients, 20 from the calcium dobesilate group and 21 from the placebo group. The two groups, at the start of this trial, were matched as regards sex, age (mean ± SD, 54 ± 5.8 years in the calcium dobesilate group and 52.5 ± 7.2 years in the placebo), duration of diabetes (mean ± SD, 10.7 ± 6.1 years in the calcium dobesilate and 9.1 ± 4.4 years in the placebo group) and diabetic control.

Retinopathy grading

After maximal dilation of the pupil, red-free fundus photographies were taken from both eyes on fields 1 and 2 as defined by the Diabetic Retinopathy [7]. A solution of 20% sodium fluorescein (14 mg per kg body weight) was then injected intravenously. The fluorescein angiography was performed according to the following protocol. A series of 20 photographies of field #2 of the worse eye was started before capillary phase of the angiogram. A sequence of 4 photographies from field #2 was also performed after 50 seconds. At 1 minute, stereophotographs of field #1 and #2 were taken on both eyes. Late photographs (10 minutes) were taken of the chosen eye.

Two parameters were evaluated on fluorescein angiography, microaneurysms and capillary closure, according to the semiquantitative grading system [15] refered to table 1 and this only on worse eye. The evaluation was performed by a senior member who did not know to whom the angiograms belonged.

Table 1. Grading system (Field #2)

Microaneurysms (MA)	Capillary Closure (CC)
0 – Absent or Questionable	0 – Absent or Questionable
1 – 1 to 5 MA	1 – < ½ Optic Disk
2 – 5 to 10 MA	2 – ½ to 1 Optic Disk
3 – > 10 MA	3 – > 1 Optic Disk

Vitreous fluorophotometry examination

The measurements were made with a commercial fluorophotometer, the Fluorotron Master TM. Details of the procedure have been previously described [4]. Scans were taken before the administration of 14 mg of fluorescein per kg and 60 minutes after injection. The fluorophotometric data were saved on magnetic diskettes and then processed to correct the influence of the autofluorescence and to provide the numerical values of the amount of fluorescein that had penetrated into the vitreous. The correction procedure was limited to of the pre-injection scan [6]. This subtraction minimises the contribution of natural fluorescence, mainly that of the lens.

As previous experience had shown that the values between 2 and 4 mm in front of the chorioretinal peak are virtually uninfluenced by chorioretinal spread function, we estimated the mean of the measurements made between 2 and 4 mm to obtain posterior vitreous values (PV2–4).

We then proceeded to determine the penetration ratio (PR) which reflects the blood-retinal barrier permeability by using the following formula:

$$PR = \frac{PV_{2\text{–}4(60')} - PV_{2\text{–}4(0')}}{\text{Plasma fluorescein integral } (3\text{–}60')}$$

Plasma fluorescein measurements

The decay of plasmatic fluorescein was expressed as a logarithmic function: log(plasma concentration) = a + b log (T), where a and b are constants that can be determined by two or more measurements, and T is the time after injection. All the technical procedures have been described in detail elsewhere [4]. We collected three blood samples on which we performed the three measurements at 10, 15 and 50 minutes.

Statistical analysis

Statistical evaluation of the fluorophotometric data was done by use of the Anova and Manova tests separately for the right and left eyes and for the worse eye from each patient. The fluorometric values at the start of the trial were correlated between both eyes in the some patient and with the values at 6 and 12 months. Therefore, the results obtained in one eye are not independent from the other and the possible difference between treatment has to be settled on one parameter in one eye. Two cases were discarded from the analysis because the values of both parameters PR and PV were widely outlying and probably corresponded to special situations. The angiographic data (microaneurysms and capillary closure) gradings from the worse eye in each patient were analysed by non-parametric tests. The laboratory data were also analysed.

Results

Permeability of the Blood-Retinal Barrier

The evolution of the PR in the calcium dobesilate and placebo groups is shown in Figs. 1 and 2. At the beginning of the study there was no statistical difference in PR between the groups, but during the study the PR in the placebo group increased markedly compared with that in the treated group and the differences were statistically significant. At 6 months $p = 0.07$ right eye, 0.02 left eye; at 12 months $p = 0.05$ right eye, 0.09 left eye; and $p = 0.025$ for the worse eye throughout the study. Thus, calcium dobesilate has a stabilising effect on the PR in all the subgroups analysed.

The normalised posteriour vitreous fluorophotometric values showed similar differences between the calcium dobesilate and placebo groups in favour of calcium dobesilate: at 6 months $p = 0.09$ right eye, 0.02 left eye; at 12 months $p = 0.04$ right eye, 0.06 left eye; and $p = 0.054$ for the worse eye over 12 months.

Microaneurysms and capillary closure

The microaneurysms and capillary closure gradings, for the more severely involved eye, did not show significant differences between the two groups at the start of the trial, but at 12 months the

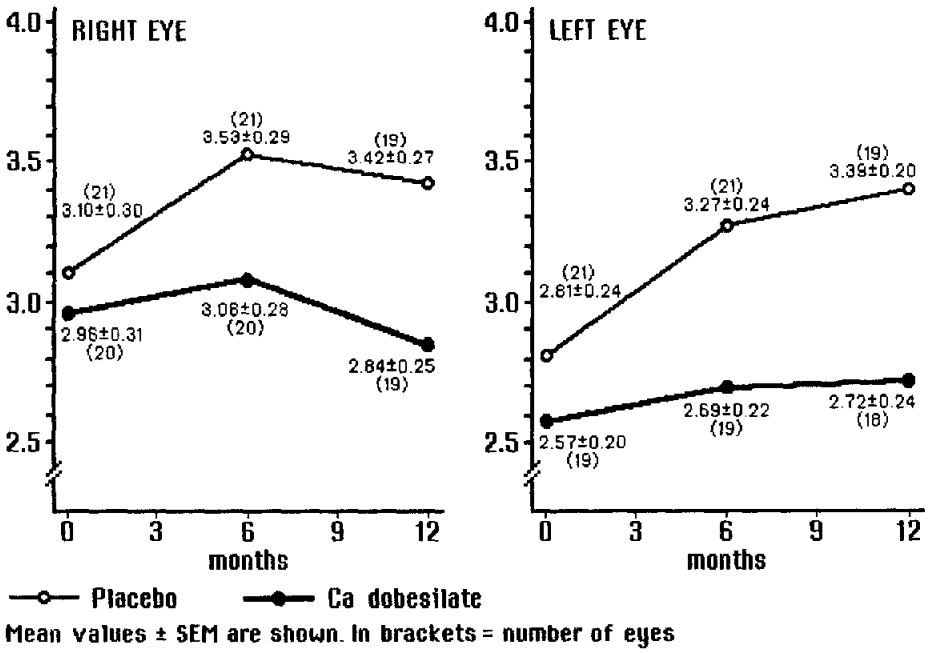

Fig. 1. Penetration ratio of the right and left eyes for 41 adult-onset diabetics treated for 12 months with calcium dobesilate (20) and with placebo (21). The penetration ratio, obtained by dividing the posterior vitreous fluorescein concentration by the integral of the plasma fluorescein concentration, is an indicator of the integrity of the blood-retinal barrier.

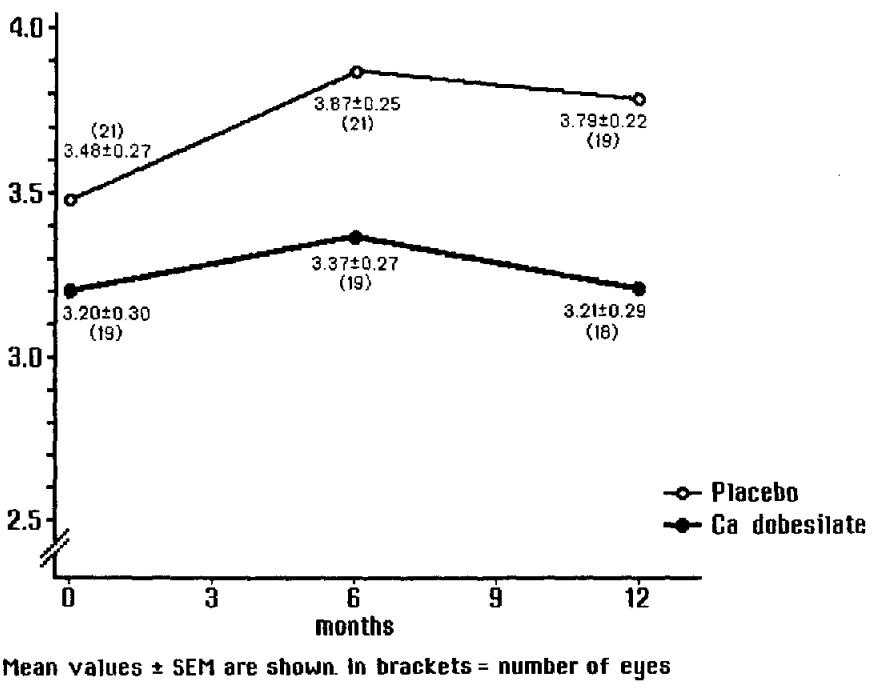

Fig. 2. Penetration ratio for the worse eye in the same patients.

following changes were recorded: the grading of microaneurysms increased in five eyes (25%) in the calcium dobesilate group and in eight eyes (38%) in the placebo group. It decreased in two eyes (9.5%) in the calcium dobesilate group and in one eye (5%) in the placebo group. The grading of capillary closure became worse in one eye (5%) in the calcium dobesilate patients and in four (19%) in those treated with placebo. The other eyes remained without retinopathy or unchanged (Table 2).

Laboratory data

No significant differences between the two groups were detected at the start and during the study (Table 3).

Side effects

No side effects were registered during the whole study.

Discussion

This study has demonstrated that calcium dobesilate administered orally in a dosage of 1000 mg. twice daily over a period of 12 months to patients suffering from adult-onset diabetes with minimal or no retinopathy has a statistically significant beneficial effect on the alteration of the blood-retinal barrier. Stabilization of blood-retinal barrier permeability in the patients given calcium dobesilate, and deterioration of the blood-retinal barrier in those given placebo, were shown by vitreous fluo-

Table 2.

Pat.	Placebo Group				Pat.	Calcium Dobesilate Group			
	0 month		12 months			0 month		12 months	
	C.C.	MA	C.C.	MA		C.C.	MA	C.C.	MA
# 2	1	3	1	3	# 1	1	3	1	3
# 3	1	1	1	1	# 5	0	1	0	1
# 6	0	0	0	1	# 7	0	0	0	0
# 8	1	3	2	3	#11	0	1	0	0
#10	1	3	1	3	#13	0	1	0	1
#14	0	0	0	1	#16	0	1	0	1
#15	0	0	0	1	#18	0	0	0	0
#17	0	0	0	1	#19	1	3	1	3
#20	1	3	2	3	#21	1	1	1	1
#22	0	1	0	0	#24	0	3	1	3
#25	0	1	1	0	#27	0	1	0	2
#28	0	2	0	2	#29	1	1	1	3
#30	0	1	0	3	#31	0	0	0	1
#32	1	2	1	2	#34	0	3	0	3
#33	0	1	0	0	#36	0	0	0	1
#35	0	1	0	2	#38	0	3	0	3
#37	0	1	2	2	#39	0	1	0	1
#42	0	2	0	2	#41	0	0	0	0
#44	0	3	0	3	#43	0	1	0	1
#45	0	0	0	0	#46	0	0	0	1
#47	0	0	0	1					

Pat. – Patient
C.C. – Capillary Closure
MA – Microaneurysm

rophotometry. Although vitreous fluorophotometry values were slightly lower in the calcium dobesilate group at the beginning of the study the differences with the placebo group were not statistically significant to presume a possible influence on the results.

The changes in microaneurysms and capillary closure which are very difficult to quantify, particularly the former because of their variableness, were, for the period of the study, minimal and did not reach the level of statistical significance.

The laboratory data on metabolic control during the period of study did not show any statistically significant differences between calcium dobesilate and placebo groups. There is, however, a suggestion that diabetic metabolic control may have been slightly more favorable in the calcium dobesilate group.

Table 3. Laboratory data in 41 adult-onset diabetics treated for 12 months with calcium dobesilate (20) and with placebo (21).

Variable	Treatment		0 month	3 months	6 months	12 months
Haemoglobin	D	m	14.53	14.40	14.21	14.20
(g/dl)		s	0.28	0.27	0.24	0.27
<11.5–16.5>	p	m	14.70	14.88	14.63	14.78
		s	0.25	0.25	0.23	0.29
Haemoglobin	D	m	9.774	9.850	9.490	9.510
A1		s	0.41	0.47	0.36	0.34
(% total Hb)	p	m	9.235	9.550	9.460	9.610
<5–8>		s	0.40	0.38	0.41	0.36
W.B.C.	D	m	6.94	6.30	6.73	7.11
(10^3/mm 3)		s	0.36	0.30	0.33	0.34
<4–11>	p	m	6.76	6.65	6.96	7.00
		s	0.23	0.28	0.29	0.27
R.B.C.	D	m	4.75	4.75	4.74	4.76
(10^6/mm 3)		s	0.10	0.12	0.11	0.11
<3.8–4.8>	p	m	4.77	4.82	4.74	4.80
		s	0.09	0.10	0.09	0.08
Urine sugar	D	m	6.09	4.57	4.57	3.93
(mmol/24 h)		s	0.97	0.81	1.14	0.82
<0–6>	p	m	5.65	6.96	6.48	5.23
		s	0.89	1.00	1.21	0.89
Blood sugar	D	m	195.2	175.9	184.9	193.2
(mg/dl)		s	11.5	11.5	11.8	13.3
<80–100>	p	m	202.7	209.6	201.3	201.0
		s	11.6	15.2	10.4	12.9
Creatinine	D	m	0.852	0.970	0.971	0.905
(mg/dl)		s	0.034	0.038	0.046	0.044
<0–1.2>	p	m	0.788	0.857	0.809	0.809
		s	0.033	0.026	0.029	0.017

( ) : units
< > : range of normal values
D : DOXIUM or calcium dobesilate
p : PLACEBO
m : mean
s : standard error of the mean

The study has confirmed that vitreous fluorophotometry is necessary in clinical trials for early diabetic retinopathy. It is the only method sensitive enough to quantify within a short time and in a reproducible manner one of the initial pathological changes of that condition. Fundus photography and fluorescein angiography which are widely used have limitations in short trials. Fundus photography does not detect leakage or capillary closure and it may not always differentiate microaneurysms from small haemorrhages. Although fluorescein angiography does demonstrate leakage and capillary closure, it does not appear to be sensitive enough to demonstrate significant differences during a trial of twelve months duration.

The mechanism whereby calcium dobesilate stabilises changes in the blood-retinal barrier which are one of the earliest features of diabetic retinopathy is not yet elucidated. Calcium dobesilate has many actions. It inhibits aldose reductase [12], prostaglandin synthetase [8, 14] and platelet activating factor [1], it has antiplatelet activity [10] and it lowers blood viscosity [11, 17]. This multifactorial activity may be especially important in diabetic retinopathy, itself apparently a multifactorial disease in which a variety of pathophysiological mechanisms appear to operate concurrently.

Calcium dobesilate's stabilising influence on the blood-retinal barrier (especially if it proves to be a sustained influence), its multifactorial action, and its absence of side effects hold out promise for its use in the early stages of diabetic retinopathy.

We realize however, that this report describes a relatively short, one year, pilot study on the effect of calcium dobesilate on early diabetic retinopathy.

Further studies, above all randomised clinical trials, over longer periods of time are called for. These, together with the development of new methods for early detection of retinal vascular changes, will improve our understanding of this critical stage of diabetic retinal disease and will help in identifying effective preventive and therapeutic agents.

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