

A preliminary trial to determine whether prevention of dark adaptation affects the course of early diabetic retinopathy

GB Arden¹, MK Gündüz², A Kurtenbach³,
M Völker⁴, E Zrenner³, SB Gündüz⁵, Ü Kamis²,
BT Öztürk² and S Okudan²

CLINICAL STUDY

Abstract

This study was designed to determine whether a new form of treatment of diabetic retinopathy (DR) was acceptable to patients and whether reduction in the maximal activity of rods in diabetes could affect the progress of DR.

Methods In 12 patients, trans-lid retinal illumination of one eye was employed during sleep to prevent the depolarisation of rods and thus reduce their metabolic activity.

Techniques A headband was used to place a source of chemical light over one eye, with its fellow as a control.

Measurements Colour contrast thresholds were measured before and after a period of treatment in treated eyes, and the changes were compared to those in untreated fellow eyes, and areas of 'dark retinal anomalies' (microaneurysms, dot haemorrhages) were measured at the same time points.

Results Patients found this intervention to be acceptable, and no adverse effects were noted. In the majority of cases, and for each outcome measure, the treated eyes improved relative to their fellows. The intervention significantly reduced the tritan thresholds in treated eyes relative to their fellows ($P = 0.03$), and the area of dark retinal anomalies decreased in treated eyes and increased in untreated eyes, with a similar probability.

Conclusions The study showed that this intervention is safe. Although the study was not powered to study efficiency, the results are promising and consistent with other reports that indicate the retina in DR is suffering from hypoxia; however, further trials should be undertaken.

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Introduction

One of the leading causes of blindness worldwide is diabetic retinopathy (DR). The earliest phase, seen in animals, is the overproduction of glial fibrillary protein in Müller cells, but soon after retinal capillary endothelial cells and pericytes show immunocytological changes, and increased TUNEL staining (indicative of apoptosis). Capillary leakage and leucostasis develop. In humans, the earliest clinical signs consist of microaneurysms and later, occlusion of small parts of the retinal capillary network. The small blood vessels of the retina are particularly sensitive to damage.¹ The most commonly accepted cause of DR is oxidative stress,¹ but it has also been suggested that other conditions contribute, notably hypoxia,² and such retinal hypoxia, must be greatly augmented during dark adaptation,^{3–5} when the rod dark current becomes maximal.^{6–9} If so, prevention of dark adaptation should slow or prevent the development of DR. This hypothesis can be tested by sleeping in an illuminated environment because this is the only time most of us ever dark adapt. To ensure constant and known retinal illumination we have devised 'light masks', which contain local light sources and used them in a clinical trial.

Materials and methods

The work was performed in accordance with the Helsinki agreement. Each patient gave informed consent.

¹Department of Optometry and Visual Science, School of Community and Health Sciences, City University, London, UK

²Department of Ophthalmology, Meram Medical Faculty, Selçuk University, Konya, Türkiye

³Centre for Ophthalmology, Institute for Ophthalmic Research, University of Tübingen, Tübingen Germany

⁴Centre for Ophthalmology, University Eye Hospital, Tübingen, Germany

⁵Department of Analytical Chemistry, Faculty of Science, Selçuk University, Konya, Türkiye

Correspondence: GB Arden, Department of Optometry and Visual Science, School of Community and Health Sciences, City University, Northampton Square, Islington, London EC1 V0 HB, UK
Tel: +44 0207 040 8863;
Fax: +44 0207 040 8355.
E-mail: g.arden@city.ac.uk

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Patients

Six male and 6 female diabetic patients were recruited. Two had type-II diabetes and were aged 65 at the beginning of the trial. The remainder had type-I with an acute onset at ages ranging from 2 to 46 years. The duration of diabetes was 5–33 years, with a mean of 16.3 years. The mean age at the start of the trial was 40.4 years. All patients had a corrected visual acuity of 20/20 or equivalent (two wore spectacles) that did not alter during the trial. All eyes had mild-to-moderate non-proliferative DR. One eye (at random) was selected for illumination. The fellow eyes were used as controls. All subjects were well controlled metabolically. Their systemic treatment was not changed during the trial.

Fundus examinations

Two-field 50-degree standard colour fundus photography for diabetic screening was performed in Turkey. The camera angle in Germany was 30 degrees. Quantitative measurements were performed on the digitised fundus photographs using the facilities of AutoCAD. The total lesion areas of ‘red dots’ were measured in a circular area of retina centred near the fovea. The diameter was about six disc diameters. The measurements were made on greatly enlarged images and lesions as small as approximately 22µm in diameter (6’ arc) could be measured. This procedure corresponds to methods employed in the measurement of drusen (in ARM)¹⁰ and various automated systems, which are designed to diagnose early DR.^{11–13} The measurements were made on images obtained before and after the patient wore the light mask. The German patients wore the light masks for 1 year. In Turkey, the duration was 3 months. The grader of the images was masked with respect to which eye was treated.

Colour contrast discrimination

Colour contrast discrimination was determined with ChromaTest, a computerised method for measuring colour contrast discrimination,^{14,15} using a 4.5-degree subtense optotype defined by chromatic difference with the background ($x=0.33, y=0.33$), with dynamic masking luminance noise of up to 40% luminance contrast. The chromaticity varies along a protan or tritan colour confusion line, chosen to be orthogonal. The maximum possible chromaticity difference determined by the monitor phosphor emissions is defined as 100%, and the threshold is expressed as a fraction of this displacement. Test–retest studies show that the threshold of an individual with no eye disease can be reproduced to within 1% (Figure 1a), and did not vary for a normal observer during the course of the trial.

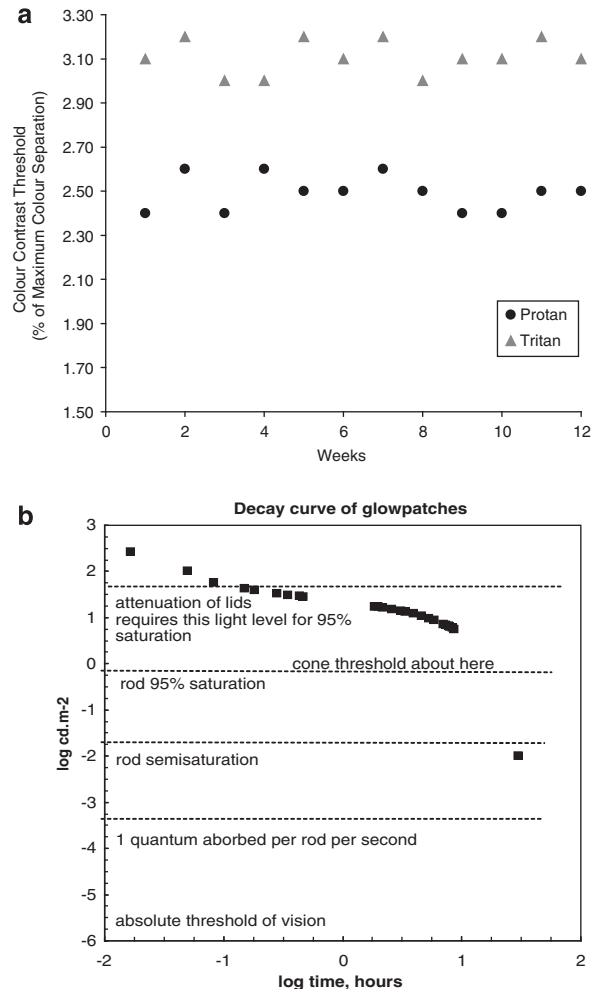


Figure 1 (a) Colour contrast thresholds of normal subject during the trial along the protan and tritan axis. Repeated measurements show no significant change during the course of the trial, and indicate the precision of measurement. (b) The decay curve of the glowpatches used. Note the logarithmic scales. The significance of the horizontal dotted lines is explained in the text.

Light sources

Light was provided by ‘glowpatches’. These safety-certified flat oval sachets, 75 × 55 mm, are made from inert flexible plastic sheeting containing a dilute solution of phthalates and hydrogen peroxide, together with proprietary catalysts. The reactants (separated by a foil partition, which could be broken by manual compression) mix and emit light of peak output 530 nm. The dominant wavelength was calculated to be 550 nm after passage of the lids. These sachets were held in place by a light plastic head band that itself was made of inert, medically approved material. Since intense blue and blue–green light is known to have toxic effects on the

retina,¹⁵ two of the non-diabetic authors in the study (MK Gündüz and GB Arden), who have no known ocular disease, used these patches in preliminary experiments on one eye during sleep—in the case of MK Gündüz for 3 months (Figure 1a). Figure 1b also shows the decay of light output with time in these patches.

Results

Safety issues

All patients enrolled in the study used the light sources uninterruptedly and none of them had any ocular or systemic complications during the study. After an initial trial lasting 1 week, the patients found they were able to sleep comfortably while wearing the mask, with a patch over one eye, and there were no drop-outs. None of the patients had any unusual symptoms and all of them were content to be in the trial, in fact some continued after the end of the trial.

Colour vision changes

The changes in the outcome measures are shown in Table 1 and Figure 2.

Two of the patients (one in each country) had very high tritan thresholds. In one case, this was due to an

old haemorrhage, which resolved during the trial, causing a large decrease of threshold. In the other, the patient had extremely high tritan thresholds (approximately 70%) and although the treated eye improved, we could not exclude some congenital or acquired pathology causing the increase. These two patients were removed from the analysis. Protan thresholds were normal in the eyes of all the remaining patients, and remained unchanged during the trial. Figure 2a shows the mean values and Figure 2b shows the changes with the standard errors. If treated and untreated eyes behaved identically, then the value presented in this figure would be zero, and the variance gives an indication of the uncertainty (noise) of the measurements. It can be seen that intervention caused no significant change.

Tritan thresholds behave in a quite different way. In 9 of 10 patients the difference between the treated and untreated eyes increased, and the figure shows that the change was mostly due to increase in threshold, which did not occur in treated eyes. The magnitude of the change was significantly different to zero by *t*-test. If the results of the two excluded patients were included, 11 of the 12 treated eyes improved relative to their fellows. The magnitude of the average improvement increased, but the variance also increased.

Table 1 Analysis of changes in colour vision

ID	Tritan results							Protan results						
	Treated eye		Untreated eye		A-B	C-D	(A-B)-(C-D)	Treated eye		Untreated eye		A-B	C-D	(A-B)-(C-D)
	Start A	End B	Start C	End D				Start A	End B	Start C	End D			
1	10.7	8.7	8.1	11.1	2.0	-3.0	5.0	3.8	3.1	5.6	5.6	0.7	0	0.7
2	4.9	4.4	5.8	4.0	0.5	1.8	-1.3	2.7	2.9	2.9	2.2	-0.2	0.7	-0.9
3	5.9	5.5	9.9	9.6	0.4	0.3	0.1	2.3	3.6	3.1	3.1	-1.3	0	-1.3
4	6.0	6.8	8.4	10.7	-0.8	-2.3	1.5	3.1	3.2	3	3.3	-0.1	-0.3	0.2
5	5.5	7.3	5.2	7.7	-1.8	-2.5	0.7	3.1	3.2	3.3	3.8	-0.1	-0.5	0.4
6	8.1	7.4	25.2	28.8	0.7	-3.6	4.3	2.2	3.5	2.2	3.5	-1.3	-1.3	0
7	5.7	5.5	5.5	7.1	0.2	-1.6	1.8	3.8	2.2	4	2.5	1.6	1.5	0.1
8	5.8	5.6	6.1	6.2	0.2	-0.1	0.3	2.3	2.4	2.3	2.4	-0.1	-0.1	0
9	8.8	8.6	8.6	8.5	0.2	0.1	0.1	3.6	3.4	3.5	3.3	0.2	0.2	0
10	5.5	5.6	5.6	6.2	-0.1	-0.6	0.5	2.1	2	2	2	0.1	0	0.1
Mean	6.7	6.5	8.8	10.0	0.2	-1.2	1.3	2.90	2.95	3.19	3.17	-0.05	0.02	0.02
SD	1.9	1.4	6.0	7.0	1.0	1.7	2.0	0.67	0.56	1.05	1.04	0.85	0.73	0.73
SE	0.6	0.5	1.9	2.2	0.3	0.5	0.6	0.21	0.18	0.33	0.33	0.27	0.23	0.23

A-B is +ve: The treated eye thresholds decrease over trial

C-D is -ve: The untreated eyes' thresholds increases over trial, approaching significance

<i>p</i> (A differs from B) by chance	=	0.641	<i>p</i> (A differs from B) by chance	=	0.857
<i>p</i> (C differs from D) by chance	=	0.063	<i>p</i> (C differs from D) by chance	=	0.933
<i>p</i> (((A-B)-(C-D))>0) by chance	=	0.03	<i>p</i> (((A-B)-(C-D))>0) by chance	=	0.359

The bold face indicates the patient who did not improve under the intervention.

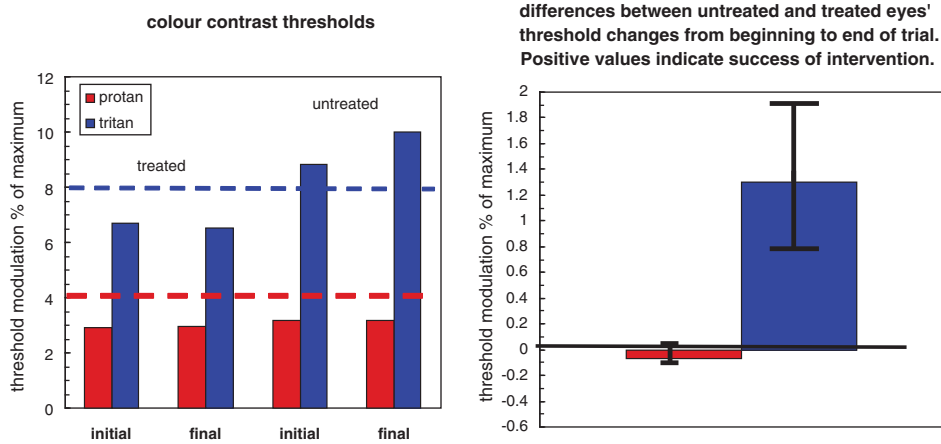


Figure 2 Graphical representation of the data shown in Table 1. Left panel: During the trial protan colour contrast thresholds did not change, but the tritan thresholds rose in untreated eyes. The horizontal lines indicate the upper limits for normal thresholds. Right panel: The difference between before and after-intervention readings for the two eyes. Positive values indicate success of the intervention in preventing rise in colour contrast threshold. The small changes for protan indicate the ‘noise’ of the measurements. The changes in tritan values are significant.

Table 2 Analysis of changes in area of retinal lesions

ID	Area of dark retinal anomalies—% of total retinal area						
	Treated eye			Untreated eye			A–B–C + D
	A Initial	B Final	A–B Change	C Initial	D Final	C–D Change	
3	0.936	0.465	0.471	0.259	0.634	–0.375	0.846
1	0.264	—	—	0.497	0.731	—	—
2	0.988	0.346	0.642	0.279	1.006	–0.727	1.368
5	—	0.329	—	0.060	0.619	—	—
11	9.691	5.203	4.488	5.203	4.825	0.379	4.109
5	0.549	0.326	0.224	0.295	0.740	–0.445	0.668
6	0.744	0.316	0.428	0.277	0.265	0.012	0.416
6	0.214	0.068	0.146	0.414	1.037	–0.623	0.769
12	0.395	0.445	–0.050	0.240	0.475	–0.235	0.185
8	0.176	0.171	0.005	0.051	0.022	0.029	–0.024
7	0.190	0.232	–0.042	0.611	0.654	–0.043	0.001
10	0.307	0.154	0.153	0.059	0.022	0.037	0.116
<i>n</i>	11	11	10	12	12	10	10
Mean	1.31	0.73	0.65	0.69	0.92	–0.20	0.85
SE	0.84	0.45	0.43	0.41	0.37	0.11	0.39
Binomial probability that 9 of 10 treated eyes improve relative to fellows =							0.01
<i>t</i> -test that magnitude of change (A–B–C + D) > 0 =							0.03

The bold face indicates the patient who did not improve under the intervention.

Fundus photography

The fundal appearances of the patients’ eyes varied considerably at the beginning of the trial. In only three were there significant exudates, and therefore change in exudates as a function of time were not analysed. The area of microaneurysms and

haemorrhages varied between individuals by a factor of 20:1, a much greater factor than that observed for colour vision variations (compare Table 1 with Table 2). In only 10 patients were complete records obtained for both eyes. The area of dark anomalies (and the numbers) decreased in the treated eyes over the course of the trial ($P=0.07$ by *t*-test), while the areas of dark

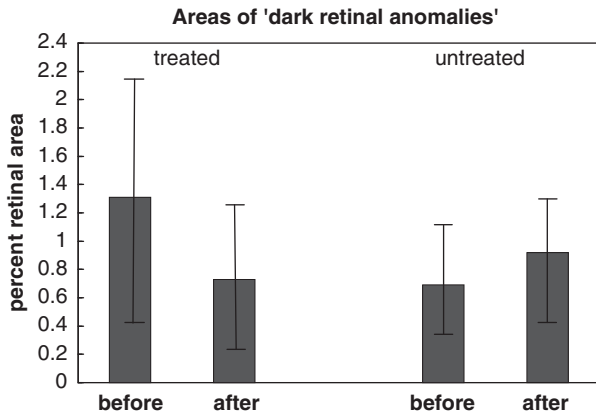


Figure 3 The percentage of retinal area occupied by microaneurysms and dot/blot haemorrhages in the patients eyes before and after treatment.

anomalies increased in untreated eyes (Table 2). In 9 of 10 cases the treated eye appeared to improve more or deteriorate less than the untreated eye. The binomial probability that the intervention was ineffectual and that the asymmetry is due to chance is 0.01. The probability (*t*-test) that the difference in column heads ($A-B-C+D$) is greater than zero is 0.03. All 10 useable records are included. Figure 3 shows the mean of all values obtained before and after the intervention in all treated and untreated eyes for which data were available.

Discussion

Although two of the authors conducted preliminary observations on themselves, this is essentially a phase-I trial, and it has shown that patients can be prevented from completely dark adapting for periods of up to 1 year without any apparent adverse effect. Also, it has been shown that this form of treatment is acceptable by patients.

Changes in colour vision

Phase-I trials do not usually provide evidence about efficacy, but our work indicates that light adaptation reduced the severity of tritan colour vision disturbance in DR. Nine of 10 (or 11 of 12 if the outliers are included) patients' treated eyes improved relative to their untreated fellows. The binomial probability that this could occur by chance is 0.01. Table 1 suggests that the major effect of treatment was to halt the deterioration found in the untreated eyes. The magnitude of the difference between eyes is also significant by *t*-test (Table 1) The absence of any effect on protan thresholds

implies that instrumental changes or biases did not occur. ChromaTest determines the precision with which the threshold determination is made, independently of the colour axis tested, and there are previous reports that in diabetics, blue–yellow deterioration occurs in the absence of red–green changes.¹⁶ The average thresholds in the patients were comparable to those found earlier in ophthalmic surgeons with diabetes and no visible fundus changes.¹⁶

Anatomical changes

A similar trend could be seen in the changes of fundal appearance: the areas of microaneurysms and small dot haemorrhages decreased in the treated eyes and rose in the untreated eyes. The number of such features varied very considerably from patient to patient, over a 20-fold range, as compared with 5-fold for colour vision testing, and thus a phase-II trial powered to assess the difference in outcomes is required to better understand the functional and morphological outcomes. The rate of formation and the persistence of microaneurysms has been analysed and varies considerably with age, type of diabetes, and blood sugar levels.¹⁷ The increase in time seen in untreated eyes is approximately similar to the findings of Hellstedt and Immonen,¹⁷ but the decrease in treated eyes is unexpectedly high and one explanation is that in treated eyes no new microaneurysms were formed during the trial and many disappeared. Further analysis was not conducted in view of the small sample size and the considerable difference between patients.

Light levels employed

This is the first trial that has attempted to measure the quantity of light that could protect the eye in DR. The only available devices produced an illumination that reduced significantly with time as indicated in Figure 1b. In Figure 1b the lowest ordinate level is $1 \mu\text{cd}$, an accepted value for the limit of human vision corresponding to a light level in which one rod in 100 is excited by a quantum every 100 s. Therefore, it is simple to calculate the light intensity that the patch would deliver to the opened eye through a dilated pupil. Despite uncertainties⁴ the initial value achieved by glowpatches is above mesopic levels, and therefore, light adaptation of rods to this level must have occurred for several hours. Such light adaptation would reduce rod dark current although not abolishing it, and this raises the possibility that if higher intensities of light were used, for longer periods, the modest improvement in treated eyes shown by the patients would be more obvious.

Relationship to previous work and theories of pathogenesis

The apparent success of the intervention strongly supports the hypothesis that hypoxia during dark adaptation is an important contributory cause of DR. There are a number of reports that point to the same conclusion. Thus, sleeping in the light reduces diabetic macular oedema.¹⁸ The same author also found that in the small minority of diabetic persons who have suffered from diabetes for >20 years, and yet have no retinal abnormality, no less than 62% sleep in the light.¹⁸ In addition, oxygen inhalation increases retinal function in diabetics and reduces diabetic macular oedema.^{19,20} The mainstay of surgical treatment, laser pan-retinal photocoagulation, is thought to be successful because it reduces the number of highly active photoreceptors, thus increasing the pO₂ by destroying many rods, with improvement in the oxygen supply to the remaining rods.⁹ Conditions under which rod activity is reduced also prevent DR.^{21–23} The loss of hypoxic upregulation of VEGF is found in patients with long standing diabetes and no retinal changes²⁴ In diabetic patients who have sleep apnoea, with more than five 'dips' (episodes of reduction of oxygen saturation below 90%) per night,^{25,26} the incidence and severity of DR is higher than that in control diabetic subjects. One common factor explains all these observations: the high metabolic rate of rods in darkness places a strain on the normal retina, which in diabetics is unsustainable and results in increased retinal hypoxia, stimulating the production of cytokines, especially vascular endothelial growth factor (VEGF), that contribute to DR.

Importance of the trial

DR is a serious complication of a very common disorder, and the prevalence is growing fast. Although treatment is available, it is difficult and expensive to organise screening programmes for the population at risk, and the treatment is invasive and destructive. This trial has shown that with a high degree of probability a different form of treatment is efficacious. Obviously it would be desirable to conduct larger trials, but the fact that significant results have been found with so few patients and with such a short trial, supports the hypothesis that dark adaptation plays an important role in the development of DR. It would be desirable to investigate different patient populations (for example those with diabetic macular oedema) using other measures of outcome such as central macular thickness using optical coherence tomography (OCT). The present work has raised the possibility that inexpensive, simple, prophylactic measures could be devised, which would greatly reduce the incidence of DR.

Summary

What was known before

- Increase in rod oxygen demand during dark adaptation.
- Circumstantial evidence for retinal hypoxia in diabetic retinopathy (DR).
- Pan-retinal photocoagulation (PRP) is probably effective through relief of retinal hypoxia.
- Anecdotal evidence that increasing hypoxia accelerated DR (and vice versa).

What this study adds

- Patients can sleep in light without adverse effects.
- Dim light apparently reverses or slows the progress of DR in a small, short trial; outcomes tested: alteration of colour vision and area of 'dark vascular anomalies'.

Conflict of interest

The authors declare no conflict of interest.

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