ORIGINAL RESEARCH

Citicoline and Vitamin B$_{12}$ Eye Drops in Type 1 Diabetes: Results of a 36-Month Pilot Study Evaluating Macular Electrophysiological Changes

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ABSTRACT

Introduction: Our aim was to evaluate the effects of 36 months of treatment with citicoline and vitamin B$_{12}$ eye drops on macular function in patients with type 1 diabetes (DM1) with mild signs of non-proliferative diabetic retinopathy (NPDR).

Methods: A prospective, randomized, interventional, monocentric, double-masked study was conducted. Twenty patients with DM1 were enrolled and randomly divided into two groups: the DC group (10 patients; mean age ± standard deviation 46.86 ± 8.78 years) in which one eye of each patient was treated with citicoline and vitamin B$_{12}$ eye drops (OMK2®, Omikron Italia srl, Italy, one drop thrice daily) for a period of 36 months; the DP group (10 patients; mean age ± standard deviation 47.89 ± 7.74 years) in which one eye of each patient was treated with placebo (eye drops containing hypromellose 0.3%, one drop thrice daily) for a period of 36 months. A total of 18 eyes (10 from the DP and 8 from the DC group, respectively) completed the study. In both groups, multifocal electroretinogram (mfERG) recordings were assessed at baseline and after 36 months. In mfERG analysis, the N1–P1 response amplitude density (RAD) evaluated in the 0–2.5° (ring 1), in the 2.5–5° (ring 2), in the 5–10° (ring 3), and in the 0–10° (ring 1 + ring 2 + ring 3) were considered.

Results: With respect to baseline, after 36 months of follow-up, the mfERG RADs recorded in R1, R2, R3, and R1 + R2 + R3 were significantly increased (i.e., R1 + R2 + R3 RAD from 21.552 ± 2.522 nV/degree^2 at baseline to 26.912 ± 2.850 nV/degree^2 at 36 months) in DC eyes, whereas in DP eyes they were significantly reduced (i.e., R1 + R2 + R3 RAD from 21.033 ± 3.574 nV/degree^2 at baseline to 16.151 ± 3.571 nV/degree^2 at 36 months).

Conclusions: This study indicates that patients with NPDR treated with citicoline and vitamin B$_{12}$ eye drops for a 36-month period achieved an improvement of the macular bioelectrical responses detectable by mfERG recordings. By contrast, during the same period of follow-up, patients with NPDR treated with placebo showed a worsening of the macular function.

Keywords: Citicoline; Diabetic retinopathy; Multifocal electroretinogram; Ophthalmology; Vitamin B$_{12}$

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Key Summary Points

Why carry out this study?

Although diabetic retinopathy (DR) has been studied as a microvascular disease, a dysfunction of retinal preganglionic elements, such as photoreceptors and bipolar cells, has been found as early as the microcirculatory abnormalities.

Several studies suggest that citicoline may induce an increase in function (neuroenhancement) and can prevent neurodegeneration (neuroprotection) of retinal ganglion cells, but there is a lack of information about the effects on the retinal preganglionic elements.

This double-blind, randomized, placebo-controlled, and prospective study was carried out to evaluate the effects of citicoline and vitamin B_{12} eye drops on the function of preganglionic elements located in the 10 central retinal degrees [assessed by multifocal electroretinogram (mfERG) recordings] in patients with mild signs of DR during a period of 36 months.

What was learned from the study?

In patients with DR treated with citicoline and vitamin B_{12} eye drops for 36 months, a significant increase of mfERG responses was detected, whereas in patients with DR treated with placebo a significant worsening of the same outcomes was found.

Data from this pilot study suggest that in patients with mild signs of DR, 36 months of treatment with citicoline and vitamin B_{12} eye drops induces a functional enhancement of preganglionic elements located in the 10 central retinal degrees.

DIGITAL FEATURES

This article is published with digital features, including a summary slide, to facilitate understanding of the article. To view digital features for this article go to https://doi.org/10.6084/m9.figshare.14519973.

INTRODUCTION

Diabetic retinopathy (DR) is a common preventable cause of blindness in adolescents and adults, resulting as a complication of diabetes mellitus [1]. Vascular changes of the retina and the choroid are the common features of non-proliferative diabetic retinopathy (NPDR) [2]. Indeed, in DR an early impairment of visual function may occur and can be detected by psychophysical methods such as Humphrey Matrix frequency doubling technology (FDT) [3], chromatic discrimination [4], dark adaptation, visual field [5], and contrast sensitivity perception [6], even in the absence of visual acuity reduction [7]. These methods, although providing qualitative information about abnormal physiology of the whole retina, do not allow one to identify a selective impairment of retinal elements. By contrast, electrophysiological methods permit one to objectively evaluate the function of different elements of the whole retina. In particular, in NPDR, a preganglionic (photoreceptors and bipolar cells) dysfunction can be detected by reduced flash-electroretinogram (ERG) amplitude, an involvement of amacrine cells can be revealed by reduced oscillatory potentials, and a functional impairment of the retinal ganglion cells (RGC) can be assessed by abnormal pattern-ERG responses [8].

The most appropriate electrophysiological method to assess macular function is the recording of multifocal electroretinogram (mfERG) responses that allow, by "ring analysis", one to selectively evaluate the function of preganglionic elements located in the 0–2.5, 2.5–5, 5–10, 10–15, and 15–20 degrees of eccentricity from the fovea. In the mfERG analysis, the parameter that can be considered as the most relevant to describe retinal

functional changes is the response amplitude density (RAD) [9].

In agreement with other previous reports [10, 11], we observed abnormal mfERG responses in patients with type 1 diabetes (DM1) with and without clinical signs of DR [12] and this may be relevant in clinical practice because a dysfunction of photoreceptors and bipolar cells can be selectively identified in the early stage of DR.

An important goal in NPDR can be to stabilize or reduce the retinal dysfunction detectable by psychophysical and/or electrophysiological methods. In this context, diet supplement interventions, based on medical nutrients that support conventional therapies, are known to reduce diabetes disease risk, severity, and complications, and in the case of DR to protect the retina and choroid [13].

In patients with DM1, long-term stabilization and positive changes of psychophysical macular responses (i.e., FDT 10-2 mean sensitivity) and microvascular involvement were obtained by the administration for 36 months of eye drops containing citicoline and vitamin B_{12} [14]. In addition, in patients with DR the same combination improved the morphology and sensitivity of corneal nerves [15].

Citicoline (cytidine 5′-diphosphocholine), which may act by multifactorial mechanisms of action (extensively reported in a recent published review [16]), administered in oral solution and/or in eye drops slowed down or improved the visual impairment in neurodegenerative diseases such as glaucoma [17–19] and non-arteritic ischemic optic neuropathy (NAION) [20]. It is also reported that citicoline eye drops may have neuroprotective effects also on retina and choroidal structures in experimental models of retinal neurodegeneration in vitro [21] and in a mouse model of long-lasting diabetes [22].

Vitamin B_{12} deficiency, instead, has a specific effect in promoting oxidative stress in DR and suboptimal vitamin co-factor availability also impairs the release of neurotrophic and neuroprotective growth factors [13].

At present, there is a lack of information about potential long-term functional effects on the macular preganglionic elements of patients with DM1 induced by the treatment with citicoline and vitamin B_{12}. Therefore, we aimed to study whether the treatment with eye drops containing citicoline and vitamin B_{12} could induce changes of the function of preganglionic elements in NPDR eyes after a 36-month period of follow-up. Toward this aim, this work presents the mfERG data collected at baseline and after 36 months recorded from the same cohort of patients who participated in our first pilot study [14]. The present outcomes were not included in the main study, registered at ClinicalTrials.gov as NCT04009980, and are herein presented as ancillary findings.

METHODS

Patients

A cohort of 20 patients with DM1 and NPDR [23] participated in this study; they were the same as those selected and enrolled in the main pilot study [14]. The inclusion and exclusion criteria are reported in our previously published work [14].

Study Design

This prospective, interventional, randomized, double-masked, monocentric, ancillary study (see "Introduction") was conducted according to the tenets of the Declaration of Helsinki and approved by the local IRB (Scientific Committee of Fondazione Bietti, Rome, Italy). An appropriate signed informed consent form was obtained from all patients for the mfERG evaluation at the time of recruitment.

At baseline, as previously reported [14], the enrolled patients were randomly divided into two age-matched groups: Briefly, one group of 10 patients (10 eyes, DC group) that was treated with citicoline and vitamin B_{12} eye drops (OMK2® containing citicoline 2%, hyaluronic acid 0.2%, and cyanocobalamin 0.05%; Omikron Italia srl, Italy), one drop thrice daily for a total period of 36 months; one group of 10 patients (10 eyes, DP group) who received placebo treatment (eye drops containing

hypromellose 0.3%), one drop thrice daily for a total period of 36 months.

The randomization key was opened to all investigators at the end of the follow-up.

In DC and DP eyes, mfERGs were recorded (see "Methods" below) at baseline and at the end of treatment (36 months). This allowed us to obtain reliable mfERG data from 18 patients with DM1: 10 from the DP group and eight from the DC group; two DC patients were lost at 36 months because of their unavailability.

MfERG Recordings

In DC and DP eyes, mfERGs were recorded according to the standard ISCEV [24] and our previously published method [9, 12, 25–27].

In the analysis of mfERG responses, we analyzed the average RAD (measured in nanovolt per degree^2) between the first negative peak, N1, and the first positive peak, P1, obtained in five concentric annular retinal regions (rings) centered on the fovea. We analyzed exclusively the N1–P1 RADs derived from 0 to 2.5° (ring 1, R1), from 2.5 to 5° (ring 2, R2), from 5 to 10° (ring 3, R3), and from 0 to 10° (ring R1 + R2 + R3).

MfERGs were performed two times on two different days in each patient with DM1 to assess test–retest variability. The recording with the highest R1–R3 N1–P1 RADs was considered in the statistical analysis (see below).

Statistics

Considering the aim of this ancillary study, sample size estimates were obtained from pilot evaluations of mfERG recordings performed in 10 patients with DM1 other than those included in the current study (unpublished results), using R1 RAD as main outcome measure. With a power of 90% at an alpha = 0.01, to detect an expected difference of 35% in mfERG RAD, a sample size of eight subjects was obtained.

The dropout of 20% was estimated; therefore, the number of subjects was 10 for both groups.

Test–retest data of mfERG results were expressed as the mean difference between two recordings obtained in separate sessions ± the

standard deviation (SD) of this difference. The Anderson–Darling and Kolmogorov–Smirnov tests were applied to verify that the data were normally distributed. Indeed 95% confidence limits of test–retest variability in patients were established assuming a normal distribution. In patients with DM1, test–retest data were calculated considering the entire cohort of enrolled patients that completed the study (18 DM1 eyes).

At baseline, the differences of mfERG values detected in the groups (DC and DP groups) were evaluated by one-way analysis of variance (ANOVA).

At the end of follow-up, individual changes (values detected at 6 months minus those detected at baseline) of mfERG data detected in DC and DP groups were calculated by performing a logarithmic transformation. The mean of individual changes (36 months minus baseline) of mfERG data detected in DC and DP groups was compared by one-way ANOVA.

Mean values of absolute changes in mfERG data observed in DC and DP groups after 36 months were compared to baseline values by one-way ANOVA.

All statistical analyses were performed by using MedCalc V.13.0.4.0 (MedCalc, Mariakerke, Belgium), and a p value less than 0.01 was considered as statistically significant.

This study was approved by the local IRB (Scientific Committee of Fondazione Bietti, Rome, Italy) as an ancillary outcome of the study previously registered at ClinicalTrials.gov (NCT04009980). All procedures performed in this study were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. Informed consent was obtained from all individual participants included in the study.

RESULTS

As reported in our previous study [14], the DC group comprised 10 eyes of five male and five female patients with mean age ± SD of 46.86 ± 8.78 years; mean duration of disease of
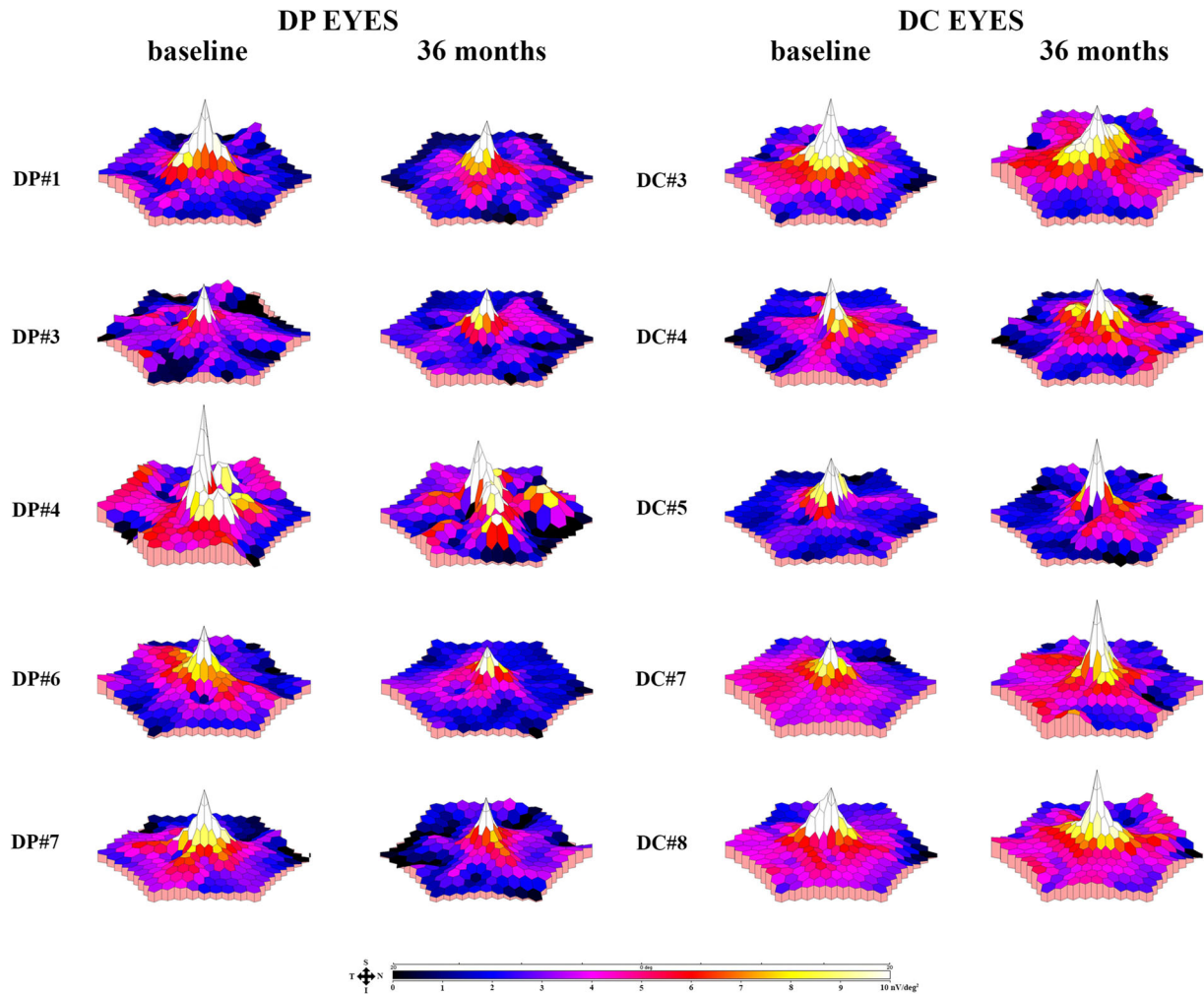
Fig. 1 Examples of three-dimensional multifocal electroretinogram (mfERG) plots recorded in representative patients with diabetes mellitus type 1 (DM1) treated with placebo (DP eyes) or with citicoline and vitamin B_{12} eye drops (DC eyes) at baseline and after 36 months of follow-up. At 36 months, with respect to baseline, the plot of DP eyes showed unmodified (DP#3 and DP#7 eyes) or worsened (DP#1, DP#4, and DP#6 eyes) central localized amplitudes, whereas DC eyes showed improved (DC#5, DC#7, and DC#8 eyes) or unmodified (DC#3 and DC#4 eyes) central localized amplitudes. Unmodified eyes are those in which the changes (36 months minus baseline) of N1–P1 response amplitude density (RAD) values were within the 95% confidence test–retest limit; improved eyes are those in which the changes of RAD values were over the 95% confidence test–retest limit; and worsened eyes are those in which the changes of RAD values were under the 95% confidence test–retest limit

23.72 ± 12.82 years; mean percentage of HbA1c of 7.721 ± 0.811; and mean best corrected visual acuity (BCVA) (by Early Treatment Diabetic Retinopathy Study [ETDRS] letter score) of 87.782 ± 4.861. The DP group comprised 10 eyes of six male patients and four female patients with mean age ± SD of 47.89 ± 7.74 years; mean duration of disease of 21.78 ± 9.42 years; mean percentage of HbA1c of 7.812 ± 0.791; and mean BCVA of 86.330 ± 5.850.

Table 1 mfERG data at baseline and during follow-up

	Baseline		ANOVA vs DP		36 months		ANOVA vs baseline	
	Mean	SD	$f(1,17)$	p	Mean	SD	f DP $(1,19)$ f DC $(1,15)$	p
R1 RAD (nV/degree^2)								
DP ($n = 10$)	70.482	7.482			51.291	10.912	21.051	< 0.001
DC ($n = 8$)	69.193	6.184	0.152	0.700	80.442	7.564	10.622	0.006
R2 RAD (nV/degree^2)								
DP ($n = 10$)	31.801	5.252			24.773	5.370	8.763	0.008
DC ($n = 8$)	31.542	3.310	0.010	0.944	37.082	2.871	12.792	0.003
R3 RAD (nV/degree^2)								
DP ($n = 10$)	19.082	3.581			14.622	2.990	9.143	0.007
DC ($n = 8$)	19.883	3.421	0.233	0.637	25.050	2.861	10.762	0.005
R1 + R2 + R3 RAD (nV/degree^2)								
DP ($n = 10$)	21.033	3.574			16.151	3.571	10.451	0.005
DC ($n = 8$)	21.552	2.522	0.061	0.803	26.912	2.850	11.144	0.005

Mean ± 1 standard deviation (SD) of multifocal electroretinogram (mfERG) N1–P1 response amplitude density (RAD) values detected at baseline and after 36 months of follow-up in patients with diabetes mellitus type 1 treated with placebo (DP eyes) or with OMK2® (DC eyes). Ring (R) refers to localized mfERG responses averaged in eccentricity areas within the macular area: 0–2.5 (R1), 2.5–5 (R2), 5–10 (R3), 0–10 (R1 + R2 + R3) degrees. ANOVA = one way analysis of variance (ANOVA) between DP and DC groups at baseline and in DP and DC groups at 36 months vs baseline; n = number of eyes

Figure 1 shows examples of a three-dimensional mfERG plot recorded in five representative DC and DP eyes at baseline conditions and after 36 months.

At baseline, DC and DP groups showed not significantly ($p > 0.01$) different R1, R2, R3 and R1 + R2 + R3 RAD values. Mean RAD values and relative statistical analysis are reported in Table 1 (baseline).

At 36 months of follow-up, in the DC group we observed that a great percentage of eyes (75% for R2 and R1 + R2 + R3 and 62.5% for R1 and R3) showed increased RAD values with respect to baseline ones, whereas in a small percentage of eyes (25% for R2 and R1 + R2 + R3 and 37.5% for R1 and R3) the RAD values were similar to baseline ones. None of DC eyes showed worsened RAD values in each considered ring.

At 36 months of follow-up, in the DP group a reduction of RAD values with respect to baseline ones in a great percentage of eyes (90% for R1, 70% for R2, and 80% for R3 and R1 + R2 + R3) was detected, whereas in a small percentage of eyes (10% for R1, 30% for R2, and 20% for R3 and R1 + R2 + R3) the RAD values were similar to baseline ones. None of the DP eyes showed an increase of RAD values in each considered ring.

The individual changes observed at 36 months in DC and DP groups with respect to baseline are reported in Table 2 and in Fig. 2.

When we considered the mean of individual changes at the end of follow-up (36 months with respect to baseline), in the DP group a mean decrease of RAD values in R1, R2, R3, and R1 + R2 + R3 was observed; by contrast DC group showed a mean increase of RAD values in

Table 2 Individual mfERG changes at the end of follow-up

	Worsened		Unmodified		Improved	
	N	%	*n*	%	*n*	%
R1 RAD						
DP (*n* = 10)	9	90	1	10	0	0
DC (*n* = 8)	0	0	3	37.5	5	62.5
R2 RAD						
DP (*n* = 10)	7	70	3	30	0	0
DC (*n* = 8)	0	0	2	25	6	75
R3 RAD						
DP (*n* = 10)	8	80	2	20	0	0
DC (*n* = 8)	0	0	3	37.5	5	62.5
R1 + R2 + R3 RAD						
DP (*n* = 10)	8	80	2	20	0	0
DC (*n* = 8)	0	0	2	25	6	75

Individual changes (36 months of follow-up minus baseline) of multifocal electroretinogram (mfERG) N1–P1 response amplitude density (RAD) values observed in patients with diabetes mellitus type 1 treated with placebo (DP eyes) or with OMK2® (DC eyes). Ring (R) refers to localized mfERG responses averaged in eccentricity areas within the macular area: 0–2.5 (R1), 2.5–5 (R2), 5–10 (R3), 0–10 (R1 + R2 + R3) degrees. Worsened = reduction mfERG RAD values that exceeded the 95% confidence test–retest limit; unmodified = mfERG RAD values within the 95% confidence test–retest limit; improved = increase of mfERG RAD values that exceeded the 95% confidence test–retest limit; *n* = number of eyes

R1, R2, R3 and R1 + R2 + R3. Thus, for each considered ring (R1, R2, R3, R1 + R2 + R3), the mean values of RAD individual changes observed in DC group were significantly ($p < 0.01$) different with respect to those detected in DP group.

The mean values of individual changes at the end of follow-up (36 months with respect to baseline) observed in DC and DP groups are reported in Table 3.

Considering the mean values at the end of follow-up (36 months) with respect to baseline, in DC group a significant ($p < 0.01$) increase of

R1, R2, R3, and R1 + R2 + R3 RAD values was observed. By contrast in the DP group, a significant ($p < 0.01$) reduction of R1, R2, R3, and R1 + R2 + R3 RAD values was detected. The mean RAD values of DC and DP groups observed at baseline and at 36 months and the relative statistical analysis (vs baseline) are reported in Table 1.

DISCUSSION

The present work, an ancillary of a pilot study already published [14], presents mfERG findings recorded from the same DM1 cohort of patients and highlights functional changes of preganglionic macular elements in those patients with NPDR, who underwent treatment with eye drops containing citicoline and vitamin B_{12} (DC group) for 36 months, comparing data to a DM1 control group treated with placebo (DP group).

Our results indicate a significant difference between the DC and DP groups at the end of 36 months of follow-up: in the DP group a significant decrease of mfERG RADs evaluated in the 0–10 central retinal degrees was observed, whereas in the DC group a significant increase of mfERG RADs evaluated in the 0–10 central retinal degrees was detected with respect to baseline data. These findings suggest that in patients with DM1 and NPDR, the treatment with citicoline and vitamin B_{12} eye drops may improve the function of preganglionic elements (photoreceptors and bipolar cells) located in the 0–10 central retinal degrees [11, 24–26] during 36 months of follow-up; by contrast, in DP eyes there is a functional worsening of preganglionic elements.

As observed by several studies performed in glaucoma [19, 28, 29] or in NAION [20, 30], the treatment with citicoline improves the function of RGC, and this can be ascribed to the multifactorial mechanisms of action of citicoline which produce neuroenhancement, neuroprotective, and neuroregenerative effects on these retinal elements (see, for reviews, Parisi et al. [31] and Faiq et al. [32] where these mechanisms are described in detail).

In the present study, for the first time, we aimed to investigate the possible functional

Individual differences (36 months minus baseline)
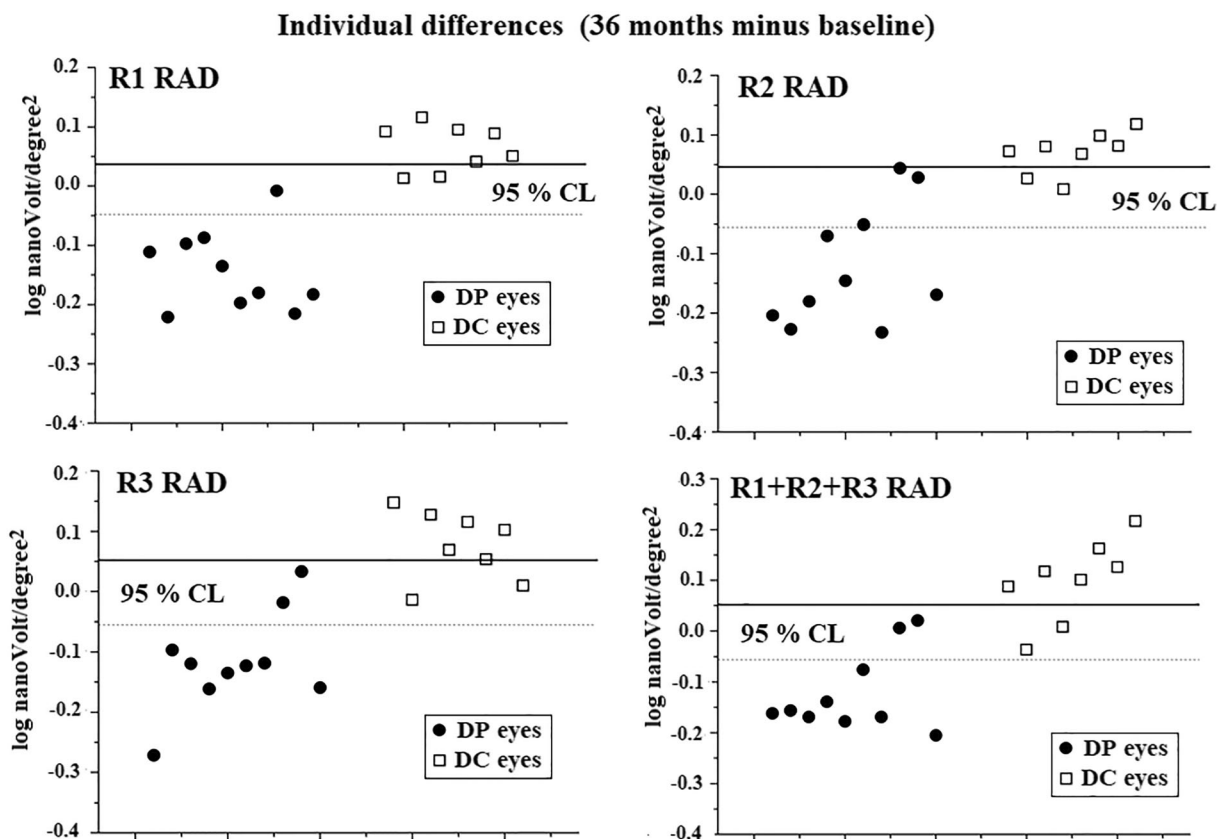
Fig. 2 Individual changes after 36 months of follow-up with respect to baseline of multifocal electroretinogram (mfERG) R1, R2, R3, and R1 + R2 + R3 N1–P1 response amplitude density (RAD) values observed in patients with diabetes mellitus type 1 treated with OMK2® eye drops (DC eyes) or with placebo (DP eyes). Ring (R) refers to localized mfERG responses averaged in eccentricity areas within the macular area: 0–2.5 (R1), 2.5–5 (R2), 5–10 (R3), 0–10 (R1 + R2 + R3) degrees. The percentage of unmodified eyes (within the 95% confidence test–retest limit), eyes with improvement (values over the 95% confidence test–retest limit; solid line) and eyes with worsening (values under the 95% confidence test–retest limit; dashed line) are reported in Table 2

effects of citicoline on different retinal elements (photoreceptors and bipolar cells) proximal to RGC.

It is noteworthy that citicoline was not administered alone but in combination with vitamin B_{12} and, therefore, the possible mechanisms of action of each component, leading to the observed functional changes, must be considered. Thus, several hypotheses should be posed to explain the functional improvement of preganglionic elements detected in DC patients.

First: Role of Vitamin B_{12} in Function of Photoreceptors and Bipolar Cells

We acknowledge that reducing oxidative stress is one of the potential therapeutic targets in DR, because patients with DM1 have a high incidence of deficiencies of crucial vitamins, minerals, and related compounds involved in the regulation of redox homeostasis. Therefore, reducing nutrient deficiencies may itself reduce the impact and severity of the disease [13]. Indeed, photoreceptors consume O_2 at very high rates compared with other cells of the organism and are thus exposed to a high degree

Table 3 MfERG differences at the end of follow-up

	Baseline		ANOVA vs DP	
	Mean	**SD**	$f(1,17)$	p
R1 RAD differences (log nV/degree^2)				
DP ($n = 10$)	− 0.144	0.068		
DC ($n = 8$)	0.064	0.039	58.872	< 0.001
R2 RAD differences (log nV/degree^2)				
DP ($n = 10$)	− 0.121	0.102		
DC ($n = 8$)	0.069	0.036	24.992	< 0.001
R3 RAD differences (log nV/degree^2)				
DP ($n = 10$)	− 0.117	0.082		
DC ($n = 8$)	0.076	0.057	31.812	< 0.001
R1 + R2 + R3 RAD differences (log nV/degree^2)				
DP ($n = 10$)	− 0.123	0.079		
DC ($n = 8$)	0.098	0.081	34.020	< 0.001

Mean ± 1 standard deviation (SD) of multifocal electroretinogram (mfERG) N1–P1 response amplitude density (RAD) individual differences (36 months of follow-up minus baseline) in patients with diabetes mellitus type 1 treated with placebo (DP eyes) or with OMK2® (DC eyes). Ring (R) refers to localized mfERG responses averaged in eccentricity areas within the macular area: 0–2.5 (R1), 2.5–5 (R2), 5–10 (R3), 0–10 (R1 + R2 + R3) degrees. ANOVA = one way analysis of variance between DP and DC groups; n = number of eyes

of reactive oxygen species (ROS) generation [33]. Furthermore, energetic metabolism of these cells is based on glycolysis and oxidative phosphorylation, which are additional sources of redox unbalance [33]. If the constitutive basal levels of redox scavenging systems, together with bulk proteolytic activity of dedicated systems [e.g., ubiquitin proteasome system (UPS) and autophagy] expressed by these cells, are enough to cope with all these metabolic insults during life, hyperglycemia, and the burden of metabolic end byproducts they bring about (AGEs, ROS, polyol pathway among the others) they probably overcome this delicate equilibrium. Over time, this is expected to lead to pathological alterations, such as those seen in

DR. In addition, the beneficial action of vitamin B$_{12}$ on the diabetic rat retina resulted from circumventing retinal hypoxia, preventing VEGF overexpression, and reducing endoplasmic reticulum stress-mediated cell death [34]. Therefore, it is likely that vitamin B$_{12}$ itself, by reducing oxidative-related processes, might induce the functional amelioration (mfERG improvement) observed in this study.

Second: Role of Citicoline in Photoreceptors and Bipolar Cells' Function

Although well-documented neuroenhancement, neuroprotection, and neuroregenerative effects of citicoline on RGC were described (see, for a review, Parisi et al. [31] and Faiq et al. [32]), similar effects on preganglionic elements are not supported by molecular insights coming from preclinical experimental models, mostly reflecting the technical and biological limitation of setting up cell cultures of these histotypes in vitro.

In this regard, it was reported that citicoline, at the level of RGC, can supply biosynthetic precursors of phospholipids, to promote ROS scavenging, mitochondrial functionality, and protection from apoptosis through inhibition of PLA$_2$ and stimulation of cardiolipin and sphingomyelin [32]. More recently, it was reported that citicoline stimulates the proteolytic activity of proteasome and the targeted proteolysis of the whole UPS, likely conferring further protection toward proteo-toxicity [33, 35, 36]. On the basis of this evidences, it can be only hypothesized that citicoline may have similar actions on other neural retinal elements, such as photoreceptors and bipolar cells. For instance, although speculative, also considering that patients with DM1 were treated with eye drops containing citicoline and vitamin B$_{12}$, the pharmacological properties of citicoline described above [32] make this molecule particularly adapted to sustain the metabolism of photoreceptors and bipolar cells which are continuously exposed to metabolic threats derived from the biological function they carry out in vivo.

Third: Combined Effects of Citicoline and Vitamin B_{12} on Outer Plexiform Layer (OPL), Inner Nuclear Layer (INL), and Microvascular Structures

The effects of eye drops containing citicoline and vitamin B_{12} on retinal structure and on microvascular elements (localized on OPL and INL) in patients with DM1 have been already investigated in our main recently published pilot study [14]. In detail, the long-term treatment (36 months) with citicoline and vitamin B_{12} induced a stabilization of OPL thickness (where the synapse between photoreceptors and bipolar cells takes place [37]), whereas a progressive thinning of the same retinal layer in patients with DM1 treated with placebo was found [14].

As already presented in the main pilot study [14], in the same cohort of patients with DM1 citicoline and vitamin B_{12} determined the stabilization of INL thickness in comparison with an increase in thickness in the long-term in the placebo-treated group possibly because of the swelling of Müller cells, meaning a possible reduction of the glia activation.

In relation to the microvasculature structure, the vessel density of the superficial capillary plexus (SCP) and deep capillary plexus (DCP) was unmodified in patients with DM1 treated with citicoline and vitamin B_{12}, suggesting a possible protection of the vessel density reduction [14]. This led us to believe that citicoline and vitamin B_{12} may have a potential role in vascular function. In fact, in vivo and in vitro studies support that citicoline has potential vascular protective effects in the brain microvascular endothelium and the mechanism of action involves protection against cell damage/apoptosis induced by calcium ionophores or hypoxia [38].

Our previous [14] and present findings together suggested that citicoline and vitamin B_{12} exert a potential effect on the entire neurovascular unit at the level of OPL/INL, with either neuronal or vascular effects in NPDR. The protection of the impairment of the entire neurovascular unit could positively affect the activity of preganglionic elements, specifically the bipolar cells that could benefit from the better metabolic support at the OPL/INL level. Indeed, DCP is located at the inner aspect of OPL and is unquestionably important for nutrition of photoreceptor synapses and other neuronal elements by regulating retinal oxygen tension [39].

In summary, in patients with NPDR, the observed functional improvement of preganglionic elements, located in the central macular $10°$, should be ascribed to several factors: the antioxidant effect of vitamin B_{12} on the retina, the hypothesized effect of citicoline on neuronal elements other than RGC, and the combined effect of citicoline and vitamin B_{12} in limiting damage at the level of neurovascular retinal structures. Moreover, by comparing data from the same cohort of patients, our mfERG data (macular function improved in the DC group and worsened in the DP group, respectively) are consistent with the previous findings obtained by FDT evaluating the mean retinal sensitivity in the same macular area (0–10 central retinal degrees) [14].

CONCLUSIONS

Our study has some limitations. The patients with DM1 were treated with eye drops containing a combination of citicoline and vitamin B_{12}, and therefore our findings cannot be attributed to the properties of citicoline alone, since vitamin B_{12} may influence the electrophysiological (mfERG) results. Thus, additional studies comparing the activity of citicoline alone versus citicoline plus vitamin B_{12} or other compounds alone are required to validate the therapeutic efficacy of these molecules and to address whether it reflects the sum of individual biological activities of the two compounds or synergy. In addition, since the mfERG recordings were performed at baseline and after 36 months of treatment, we are not able to exactly identify when there was the effects of treatment (detectable by increased mfERG RADs) first appeared during the entire period of follow-up.

The present study indicates that patients with NPDR after 36 months of treatment with citicoline and vitamin B_{12} eye drops achieved

an improvement of preganglionic macular bio-electrical responses detected by mfERG recordings. By contrast, during the same period of follow-up, patients with NPDR treated with placebo showed worsening of macular function.

Despite the fact that this was a small ancillary study, including a limited cohort of patients with DM1, to the best of our knowledge it is the first electrophysiological study on the use of available eye drops for a therapeutical approach in the early stage of DR. Our preliminary results need to be confirmed by randomized clinical trials on a larger cohort of patients with DM1 with different stages of severity of DR and with more frequent (i.e., every 6 months) assessment of the proposed outcomes.

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