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Original Article/นิพนธ์ต้นฉบับ

A Randomized, Placebo-Controlled, Double-Blind Clinical Trial of Curcuminoids in Leber Hereditary Optic Neuropathy

การศึกษาโดยการสุ่มตัวอย่างเพื่อเปรียบเทียบการรักษาโรค Leber Hereditary Optic Neuropathy (LHON) ด้วยขมิ้นชันและยาหลอก

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Abstract

Purpose: To investigate the role of curcuminoids, an antioxidant property, in vision and blood oxidative status in G11778A Leber hereditary optic neuropathy (LHON) patients.

Methods: A total of 49 G11778A LHON patients (97 eyes) were randomly assigned to receive either 250 mg of oral curcuminoids capsules (500 mg/day) or placebo capsules twice a day for 48 weeks. The visual parameters including visual acuity, Humphrey visual field, visual evoked potential and electroretinogram were assessed at 0, 12, 24, 36 and 48 weeks after the intervention. The marker for oxidative stress, malondialdehyde (MDA) in plasma, and endogenous antioxidants in erythrocytes comprising superoxide dismutase (SOD), catalase, glutathione peroxidase (GSH-Px) and total glutathione (GSH) were measured at 0, 12, 24, and 48 weeks. Adverse events were recorded.

Results: Twenty-seven (53 eyes) and 22 (44 eyes) patients were randomized to the curcuminoids and placebo groups, respectively. The two groups were comparable in age of onset, baseline VA and duration of LHON. There were no significant changes in any visual or oxidative parameters between the two groups. There seemed to be slight improvement of the mean deviation of HVF from baseline at week 36 and 48 (P = 0.027), which was statistically significant in the curcuminoids group but not in the placebo groups. A trend of reduction of erythrocyte GSH-Px, indicating improvement of oxidative status, was observed in curcuminoids but not in the placebo group. No significant difference of adverse events between both groups was found.

Conclusions: Curcuminoids at this dose did not have significant effects on the visual and blood oxidative status compared to the placebo. However, the slight improvement of HVF-MD, a trend of reduction of GSH-Px in the curcuminoids group and the safety of curcuminoids suggested that further studies with higher doses and especially in the acute stage of LHON should be considered.

Key words: Leber hereditary optic neuropathy (LHON), 11778 mutation, curcuminoids

บทคัดย่อ:

วัตถุประสงค์: เพื่อศึกษาการใช้ขมิ้นชันซึ่งมีคุณสมบัติต้านอนุมูลอิสระในการรักษาโรค Leber Hereditary Optic Neuropathy (LHON) ที่มีการกลายพันธุ์ของยีนไมโตคอนเดรียที่ตำแหน่ง G11778A เปรียบเทียบกับการใช้ยาหลอก โดยการวัดผลระดับสายตาและสารอนุมูลอิสระในกระแสเลือด

วัสดุและวิธีการ: ทำการศึกษาโดยการสุ่มตัวอย่างในผู้ป่วยโรค Leber Hereditary Optic Neuropathy (LHON) ที่มีการกลายพันธุ์ของยีนไมโตคอนเดรียที่ตำแหน่ง G11778A จำนวน 49 คน (97 ตา) แบ่งออกเป็น 2 กลุ่ม กลุ่มแรกได้รับขมิ้นชันชนิดแคปซูล รับประทานขนาด 500 มิลลิกรัมต่อวัน กลุ่มที่สองได้รับยาหลอก เป็นเวลา 48 สัปดาห์ติดต่อกันและวัดระดับสายตา ลานสายตา ชนิด Humphrey ตรวจประสาทตาและจอประสาทตาด้วยคลื่นไฟฟ้า ก่อนและหลังเริ่มให้ยา ในสัปดาห์ที่ 12, 24, 36 และ 48 สัปดาห์ในกลุ่มที่ 1 และ 2 ตรวจวัดระดับ oxidative stress ได้แก่ ระดับ malondialdehyde (MDA) ในพลาสมา ระดับ superoxide dismutase (SOD), catalase, glutathione peroxidase (GSH-Px) and total glutathione (GSH) เม็ดเลือดแดง ที่สัปดาห์ที่ 0, 12, 24 และ 48 บันทึกอาการข้างเคียงที่เกิดขึ้นหลังการให้ยา

ผลการศึกษา: จากการสุ่มตัวอย่าง มีผู้ป่วยจำนวน 27 คน (53 ตา) ได้รับขมิ้นชัน และ 22 คน (44 ตา) ได้รับยาหลอก ทำการศึกษาเปรียบเทียบ อายุที่เริ่มมีอาการ ระดับสายตาและระยะเวลาที่มีอาการโรค LHON ไม่พบความแตกต่างของระดับสายตาและสารอนุมูลอิสระ ในกลุ่มที่ศึกษาทั้ง 2 กลุ่ม ในกลุ่มขมิ้นชัน พบค่าลานสายตาที่สัปดาห์ที่ 36 และ 48 ดีขึ้นอย่างมีนัยยะสำคัญ (P = 0.027) ค่า GSH-Px ที่ลดลงแสดงถึงค่า oxidative status ดีขึ้นในกลุ่มขมิ้นชัน ไม่พบอาการแทรกซ้อนในกลุ่มการศึกษาทั้ง 2 กลุ่ม

สรุป: ผลการใช้ขมิ้นชันไม่มีผลต่อระดับสายตา และ ค่า oxidative status ในเลือด ในกลุ่มขมิ้นชัน พบค่าลานสายตาที่ดีขึ้นอย่างมีนัยสำคัญทางสถิติ GSH-Px ที่ลดลงแสดงถึงค่า oxidative status ดีขึ้นในกลุ่มขมิ้นชัน ตลอดจนเกิดความปลอดภัยจึงควรมีการศึกษาการใช้ขมิ้นชันขนาดสูงในการรักษาโรคดังกล่าวต่อไป

Introduction

Leber Hereditary Optic Neuropathy (LHON) is the most common mitochondrial genetic disease characterized by acute to subacute, nonsynchronous bilateral, painless visual loss, predominantly in young adult males.[1] The clinical findings in LHON can be divided into acute and chronic phases. In the acute phase, patients experience blurring of central vision with severe dyschromatopsia. Visual field demonstrates central or caecocentral scotoma.

The fundal appearances typically include optic disc hyperemia and swelling and microangiopathy and the optic disc becoming pale in chronic phase.[2] Visual acuity often drops to less than 6/60 in a few weeks after the onset.[1]

Visual evoked potential (VEP) shows decreased amplitude and delayed latency, while electroretinogram (ERG) is generally normal.[2,3] In LHON, an initial increase and a subsequent decrease in peripapillary retinal nerve fibre layer (pRNFL) thickness were demonstrated on spectral-domain optical coherence tomography (SD-OCT).[4,5]

Curcuminoids is a group of phenolic compounds in which curcumin is the most abundant. It has been shown to possess antioxidant properties and its efficacy has been demonstrated in many free-radical related neurodegenerative diseases.[6] Oral curcumin administration has been shown to improve oxidative stress and antioxidant parameters in serum of chronic pancreatitis and β-thalassemia/Hb E patients.[7] Curcumin was shown to directly react with reactive oxygen species (ROS).[8] Moreover, it has been shown to induce expression of cytoprotective and antioxidant proteins which improved mitochondrial function.[9]

All of the three most common primary LHON mitochondrial DNA (mtDNA) mutations have been shown to impair the biochemical function of the respiratory complex I subunit gene. leading to increased ROS production and oxidative stress during electron transport, and resulting in retinal ganglion cell loss in LHON.[10] As oxidative stress has a leading role in the pathophysiology of LHON, treatments targeted to scavenge ROS would be promising. The previous prospective randomized study by Catarino et al., 2017 suggested the benefit of Idebenone, a short-chain coenzyme Q10 analogue which is an antioxidant, or gene therapy with intravitreal injection of rAAV2/2-ND4 for visual recovery or minimizing severity of LHON.[11-13] Curcumin is another promising antioxidant and was shown to be beneficial in neurodegeneration. Therefore, this study was carried out to determine the effectiveness of curcumin on visual symptoms, electrophysiologic findings and blood oxidative stress and antioxidant parameters in G11778A LHON patients.

Patients and Methods

This was a placebo-controlled, double-blind, randomized trial. The study population consisted of all LHON patients with G11778A mutation from the Department of Ophthalmology, Faculty of Medicine Siriraj Hospital, Mahidol University. The patients were contacted in person or by phone by a neuroophthalmologist (WC). Exclusion criteria included LHON patients with other primary LHON mutation (T14484C or G3460A), pregnant women, patients with known severe systemic underlying diseases, and patients not being able to come for follow-ups. The study protocol was approved by the Siriraj Institutional Review Board (SIRB) and was

registered in clinicalTrials.gov/ NCT00528151. All the participants entered the study with informed consents.

Eligible patients were allocated to either the experimental or placebo group by unpaired random allocation and blinding with proper allocation concealment. After one-month discontinuation of medications which involved optic nerve function including coenzyme Q10, vitamin B, vitamin C, vitamin E and steroids, the subjects received 2 capsules of either curcuminoids or a placebo daily for 12 months. Each capsule was filled with turmeric extract (calculated for 250 mg of curcuminoids, which comprised curcumin, demethoxycurcumin and bismethoxycurcumin in the ratio of 1:0.3:0.1.) and quality controlled under the Good Manufacturing Practices for pharmaceutical products. The amount of curcuminoids in the capsules was tested by the GPO one year after the production and was found to be stable.

Main Outcome Measures

Baseline parameters included visual acuity (VA) by Snellen charts, mean deviation (MD) and pattern standard deviation (PSD) of Humphrey automated visual field (HVF), P100 latency and N75-P100 amplitude in flash VEP, mesopic ERG, blood oxidative stress and antioxidant. The Snellen's VA was expressed as logMAR (logarithm of the minimal angle of resolution). The HVF was performed using Swedish Interactive Threshold Algorithm (SITA) Standard 30-2. The ERG and VEP were performed using Viking Select Master Software V7.1 according to the International Society for Clinical Electrophysiology of Vision (ISCEV). Complete blood count (CBC), liver function, renal function tests and urinalyses were performed to monitor safety. The oxidative stress indicator, malondialdehyde (MDA), was measured in plasma and the antioxidant parameters comprising superoxide dismutase (SOD), catalase, glutathione peroxidase (GSH-Px) and total glutathione (GSH) were measured in red blood cells.

The same examinations and measurements as those at baseline were performed at the 12th, 24th, 36th, and 48th week visit, except for the 36th week visit, in which the oxidative stress and antioxidant parameters were not measured.

Malondialdehyde (MDA)

MDA, a product of lipid peroxidation, was measured in plasma by the method of Asakawa and Matsushita by reacting with thiobarbituric acid in acidic and boiling temperature.[14] After cooling, butanol was added and the fluorescence of the butanol extracts was measured by spectrofluorometer at 515 nm excitation and 553 nm emission.

Superoxide dismutase (SOD)

SOD activity was determined in hemolysate by a modified method based on the ability of SOD to inhibit the reduction of nitroblue tetrazolium (NBT) by superoxide anions generated by xanthine and xanthine oxidase reaction.[15]

Catalase

Catalase activity was determined by a spectrophotometric assay based on the catalytic decomposition of hydrogen peroxide.[16]

Glutathione peroxidase (GSH-Px)[17]

Briefly, the hemolysate was added into 5 mM EDTA sodium salt, 0.1 M GSH and 10 unit/ml

glutathione reductase in Tris-HCl buffer pH 8.0. The enzymatic reaction was started with 7 mM cumene hydroperoxide which served as a peroxide substrate. The rate of decrease of the absorbance at 340 nm measured by a spectrophotometer was directly indicative of GSH-Px activity.

Total glutathione (GSH)

GSH was measured by a modified method based on the GSH recycling method. GSH reacted with 5, 5'-dithiobis-(2-nitrobenzoic acid) (DTNB) to form a yellow color and was measured by a spectrophotometer.

Statistical analysis

An intention-to-treat analysis was performed including all 49 randomized patients analyzed according to group assignment. Missing data were handled using the Last Observation Carried Forward (LOCF) method, in which the last available follow-up data were used in place of the missing data.[18] For visual parameters (VA, HVF, VEP and ERG) all the examined eyes (97 eyes) were included.

Comparisons of continuous data between the curcuminoids and placebo groups were performed using two-way repeated measures ANOVA. For comparison of duration of LHON between the two groups, the Mann-Whitney U test was employed since the data were not normally distributed. Percentage changes in parameters at each visit from baseline values were calculated and compared between the two groups. Within group changes from baseline values of continuous parameters in each visit were tested using one-way repeated measures ANOVA with Bonferroni adjustment. For comparisons of nominal data, Chi-square test was used. $P < 0.05$ was considered significant. Statistical calculations were performed with SPSS for Windows 15 (SPSS Inc, Chicago, IL).

Results

Samples

Eighty-seven patients were assessed during May 2005 to December 2007 for eligibility, (Figure 1). Baseline characteristics between the curcuminoids and placebo groups were comparable (Table 1).

Visual parameters

There were no statistical differences in VA, MD, PSD, ERG amplitude, VEP amplitude and VEP latency between the curcuminoids and placebo groups at baseline and each follow-up visit. The means of percentage change from baseline of each parameter are shown in Table 2. No statistically significant differences in the means of percentage change from baseline of any parameters between the two groups were observed at any follow-up visit.

At 48th week visit, of 53 patients, 12 patients in curcuminoids group and of 44 patients, 12 patients in placebo group had improvement of VA. The proportions of eyes with VA improvement of ≥ 0.2 logMAR were similar between the two groups (P = 0.60, Chi-square test) with the overall frequency of VA improvement at 23.7%. However, in each group, when each follow-up value of the visual parameters was compared to the corresponding baseline value, there were some significant changes in the visual field. The results showed that there was subtle but statistically significant improvement of MD at week 36th (P = 0.027) and 48th week (P = 0.027) in the curcuminoids group, while the values were not significant in the placebo group. The PSD increased significantly at

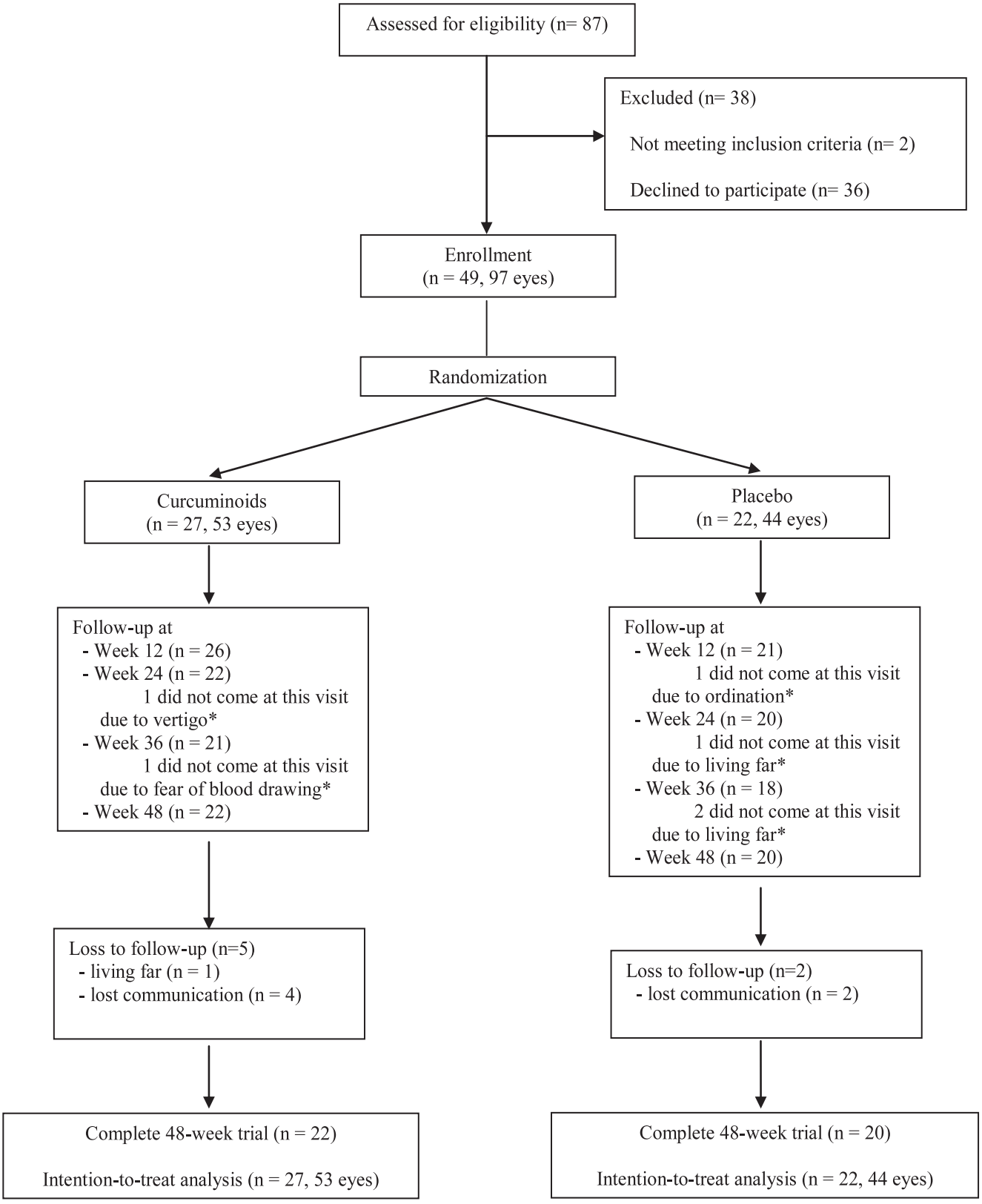

Figure 1 Flow of enrollment and follow-up of participants in the study. *, the trial medications were sent to the subjects by mail.

Table 1 Characteristics of the G11778A LHON patients in the study

Characteristics	Curcuminoids	Placebo	*P*-value
No. of cases	27	22	
No. of eyes	53	44	
Sex			0.97
Male (%)	23 (85)	19 (86)	
Female (%)	4 (15)	3 (14)	
Age (years)			0.88
Mean	32.0	31.4	
SD	14.1	12.9	
Median	27	28.5	
Range	12-67	14-62	
Age of onset (years)			0.55
Mean	22	23.7	
SD	9	10.4	
Median	20	20	
Range	16-44	7-53	
VA at baseline (logMAR)			0.99
Mean	1.85	1.86	
SD	0.76	0.52	
Duration of LHON (months)			0.37
Mean	106.7	87.6	
SD	130.8	105.8	
Median	66	46	
Range	4-475	2-370	

week 36th (P = 0.011) and 48th week (P = 0.021) in the curcuminoids group but not in the placebo group (Figure 2). The median duration of LHON before treatment in eyes that showed improvement of MD was 65 months (range 5-378 months, n = 27) and 76 months (range 2-348 months, n = 31) in the curcuminoids and placebo groups, respectively. The difference was not statistically significant (P = 0.69, Mann-Whitney test). Therefore, it was unlikely that the significant improvement of MD in the curcuminoids group was largely explained by spontaneous visual recovery seen in patients with more recent onset.

Oxidative stress and antioxidant parameters

The oxidative stress parameter, plasma MDA, and antioxidant parameters in red blood cells comprising SOD, catalase, GSH-Px and GSH, were measured before and at 12th, 24th and 48th week after treatment. The effects of curcuminoids on these parameters were not statistically different compared with the placebo (Table 3).

The levels of MDA, SOD, catalase and GSH changed in a similar fashion over time comparing between the two groups but this was not the case for GSH-Px (Figure 2). It was found that GSH-Px showed

Table 2 Means of percentage changes from baseline of each visual parameter between curcuminoids and placebo group at each follow-up visit

Parameters	Week	Mean of % change from baseline			*P*-values
		Curcuminoids	Placebo	Difference (95% CI)	
LogMAR					
	12th	-0.97	3.07	-4.04 (-10.44, 2.36)	0.21
	24th	0.02	3.12	-3.11 (-10.56, 4.34)	0.41
	36th	-4.33	-1.88	-2.45 (-11.63, 6.72)	0.60
	48th	-1.95	-3.03	1.09 (-6.86, 9.03)	0.79
HVF MD (dB)					
	12th	1.58	8.62	-7.04 (-21.00, 6.92)	0.32
	24th	0.05	1.84	-1.79 (-15.55, 11.97)	0.80
	36th	-3.98	1.02	-5.00 (-20.75, 10.75)	0.53
	48th	-3.97	1.66	-5.63 (-20.41, 9.15)	0.45
HVF PSD (dB)					
	12th	5.52	8.39	-2.86 (-14.90, 9.17)	0.64
	24th	7.34	7.78	-0.44 (-13.64, 12.77)	0.95
	36th	15.00	21.66	-6.66 (-22.26, 8.94)	0.40
	48th	14.08	21.77	-7.69 (-21.95, 6.58)	0.29
Flash VEP amplitude (μV)					
	12th	17.56	20.55	-2.99 (-20.34, 14.35)	0.73
	24th	20.75	20.32	0.42 (-17.33, 18.17)	0.96
	36th	25.80	31.55	-5.76 (-30.03, 18.52)	0.64
	48th	30.06	32.12	-2.06 (-31.28, 27.17)	0.89
Flash VEP latency (ms)					
	12th	2.16	-1.01	3.17 (-2.96, 9.30)	0.31
	24th	1.24	-1.51	2.75 (-2.12, 7.62)	0.26
	36th	0.63	1.29	-0.66 (-6.25, 4.92)	0.81
	48th	1.56	1.51	0.05 (-5.73, 5.84)	0.99
Mesopic ERG b-wave amplitude (μV)					
	12th	5.35	1.71	3.64 (-9.19, 16.47)	0.57
	24th	4.53	6.78	-2.25 (-14.57, 10.06)	0.72
	36th	8.44	4.36	4.08 (-19.74, 27.90)	0.73
	48th	9.23	5.44	3.79 (-12.06, 19.63)	0.64

LogMAR, logarithm of the marginal angle of resolution; ERG, electroretinogram; HVF, Humphrey visual field; MD, mean deviation; PSD, pattern standard deviation; VEP, visual evoked potential

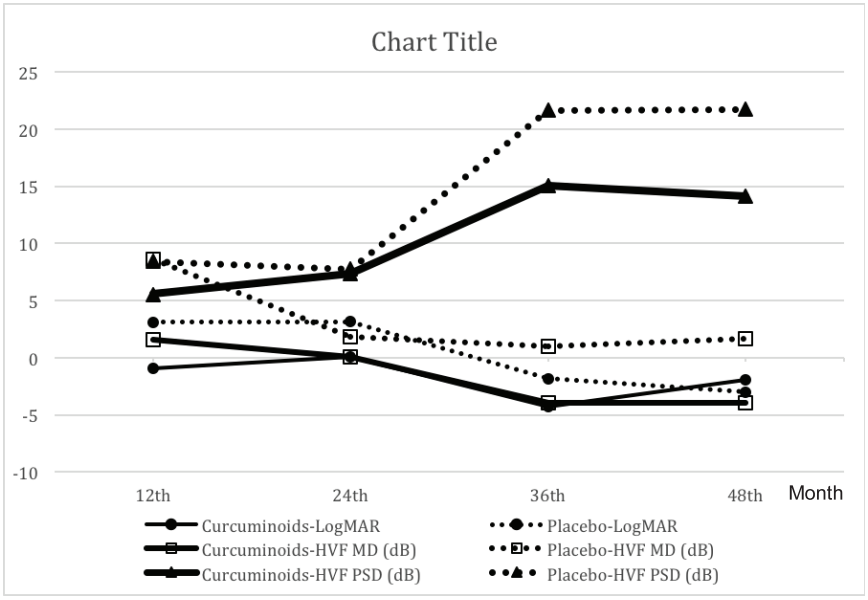

Figure 2 Shows visual acuity, visual field mean deviation (MD) and pattern standard deviation (PSD) overtime in the curcuminoids and the placebo groups. The proportions of eyes with visual acuity improvement of ≥ 0.2 logMAR were similar in the curcuminoids and the placebo groups. There was subtle but statistically significant improvement of MD and the PSD increased significantly in the curcuminoids group but not in the placebo group.

Table 3 Means of percentage changes from baseline of each oxidative stress and antioxidant parameters in blood between curcuminoids and placebo group at each follow-up visit

Parameters	Week	Mean of % change from baseline			*P*-values
		Curcuminoids	Placebo	Difference (95% CI)	
MDA (nmole/mL)					
	12th	2.91	8.69	-5.78 (-20.50, 8.93)	0.43
	24th	13.68	17.99	-4.31 (-23.91, 15.29)	0.66
	48th	23.74	27.92	-4.18 (-26.27, 17.90)	0.70
SOD (U/g Hb)					
	12th	-12.38	-11.35	-1.03 (-16.47, 14.42)	0.89
	24th	-2.37	-5.95	3.58 (-12.84, 19.99)	0.66
	48th	0.54	-0.12	0.66 (-21.81, 23.12)	0.95
Catalase (kU/g Hb)					
	12th	14.70	5.74	8.96 (-4.93, 22.86)	0.20
	24th	19.78	19.00	0.78 (-18.93, 20.49)	0.94
	48th	19.58	15.69	3.89 (-13.39, 21.17)	0.65
GSH-Px (U/L)					
	12th	-13.76	-0.41	-13.34 (-34.38, 7.70)	0.21
	24th	-8.23	14.46	-22.69 (-50.12, 4.75)	0.10
	48th	-10.93	4.27	-15.20 (-39.03, 8.62)	0.21
GSH (µmole/ g Hb)					
	12th	28.30	26.30	2.00 (-20.14, 24.14)	0.86
	24th	21.50	20.68	0.82 (-16.56, 18.19)	0.93
	48th	17.85	10.83	7.02 (-6.18, 20.22)	0.29

MDA , malondialdehyde; SOD, superoxide dismutase; GSH-Px, glutathione peroxidase; GSH, glutathione; Hb, hemoglobin

a trend of declination over time in the curcuminoids group but not in the placebo group (Figure 3). The decreased was almost significant at 12th week (P = 0.069).

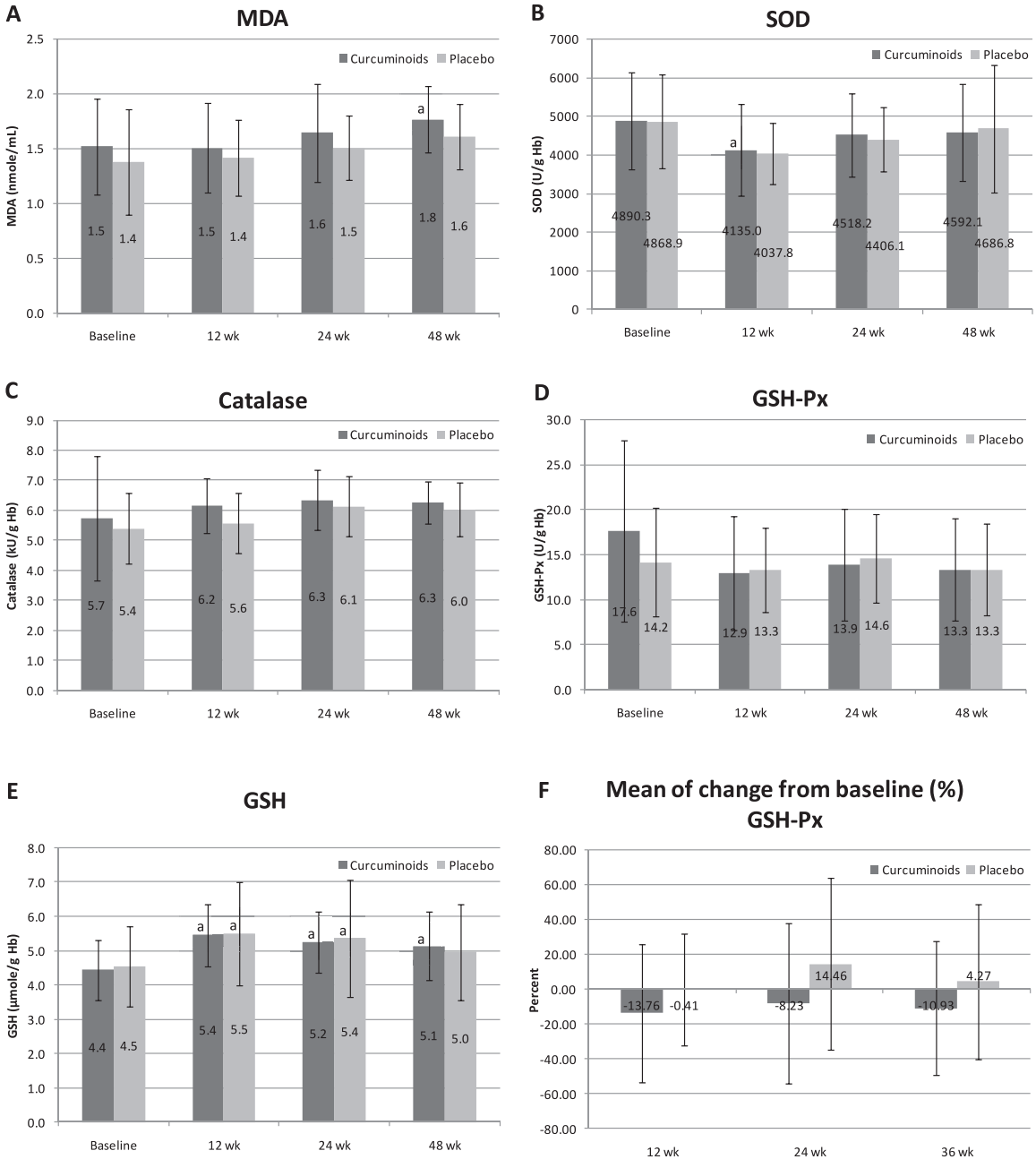

Figure 3 Bar charts showing oxidative stress and antioxidant parameters in blood of the patients in curcuminoids (n = 27) and placebo group (n = 22) at each visit. Each bar represents a mean with a standard deviation as shown by an error bar. Malondialdehyde (MDA) (A) was measured in plasma. Superoxide dismutase (SOD) (B), catalase (C), glutathione peroxidase (GSH-Px) (D) and glutathione (GSH) (E) were measured in hemolysate. The effects of curcuminoids on these parameters were not statistically different compared with the placebo. GSH-Px reduced from baseline level in the curcuminoids group but not in the placebo group (F). Hb, hemoglobin; a, statistically significant compared to baseline, using one-way repeated measures ANOVA with Bonferroni adjustment.

Compliance

Among 42 patients who completed the follow-up visit at 48th week, 22 were in the curcuminoids group and 20 were in the placebo group. All 22 patients in the curcuminoids group had more than 85% compliance to the trial medication and 77% (17/22) showed more than 95% compliance (range 87.5%–100%). In the placebo group, all 20 except 2 patients had more than 93% compliance (range 71%-100%).

Adverse effects

Dizziness, dyspepsia, diarrhea, constipation, thirst and increased appetite were reported by some subjects. There were no statistical differences in any blood parameters between the curcuminoids and placebo groups at any follow-up visits.

Discussion

Growing amount of experimental evidence revealed that increased ROS production and oxidative stress involved in the pathophysiology of LHON, secondary from the oxidative phosphorylation defect caused by the primary LHON mutation in the respiratory complex I subunit gene.[19] Thus, this study described, for the first time, the role of curcuminoids, whose antioxidant property is well-known, in LHON patients with the G11778A mutation.

This study did not find a significant effect of curcuminoids on the clinical parameters including VA, VF, VEP and ERG in G11778A LHON patients as compared with the placebo (Table 2). Similar proportions of patients in the curcuminoids and the placebo groups demonstrated some degrees of VA improvement. The overall proportion of the patients with visual improvement was 23.7%, which was in accordance with Spruijt et al., reporting 22% of G11778A LHON patients showing partial recovery of vision, and the prospective randomized study indicating the benefit of Idebenone, an antioxidant, in visual recovery[11,20] Interestingly, HVF analysis revealed a slight improvement of MD from baseline and the difference became statistically significant at 36th and 48th week in the curcuminoids group. Although there were some limitations in the HVF we performed due to the poor vision of the patients, the confirmatory fields were undertaken to minimize the variability in measuring visual function. The improvement of MD was also observed in the placebo group but it did not reach the statistically significant level. Conversely, PSD gradually increased from the baseline level and the difference became statistically significant at 36th and 48th week in the curcuminoids group, corresponding to the decrease of MD. This finding may reflect the fenestration of central scotoma, the pattern of visual recovery previously reported in LHON and curcuminoids may potentiate this improvement of vision.[11]

Oxidative status in LHON patients was investigated in blood. MDA is an indicator of oxidative stress since it is generated following oxidation of polyunsaturated fatty acids.

The results showed that curcumin did not have significant effect on plasma MDA compared to placebo. Alizadeh et al demonstrated that curcumin could be effective in reducing MDA levels but not found in our study.[21]

This was also not in agreement with a previous study in β-thalassemia/Hb E patients which showed a decrease in red blood cell MDA in the patients after treatment with 500 mg curcuminoids daily for 12

months (the same dose used in this study).[6] This might be explained by differences in the mechanism of ROS generation in thalassemia and LHON. In thalassemia, one of the major factors contributing to free radicals generation is excess iron, which is not in the context of LHON. Apart from being a free radical scavenger, curcumin possesses iron-chelating ability and thus lowering ROS in thalassemia.[22] Moreover, although G11778A is present in blood, the oxidative status in blood might not well correlate with that in the affected but inaccessible tissue, the optic nerve. This was demonstrated by the baseline level of plasma MDA of LHON patients in this study (1.46 ± 0.46 nmole/mL), which was comparable to that in healthy Thai subjects.

The antioxidant status in LHON patients were investigated by measurement of cytoprotective enzymes, namely, SOD, GSH-Px and catalase, and the endogenous cellular antioxidant GSH in red blood cells. Curcuminoids treatment for 48 weeks did not show statistically significant effects on these parameters as compared to a placebo. However, in the group treated with curcuminoids, we found some effect of curcuminoids in lowering the activity of GSH-Px which was not observed in the placebo group (Figure 2). GSH-Px and catalase are enzymes that catalyzed toxic hydrogen peroxide to oxygen and water. The increase of ROS is associated with upregulation of GSH-Px and the antioxidant ability of curcuminoids may decelerate this process, resulting in the decreased activity of GSH-Px.[23-25]

This effect of curcuminoids on GSH-Px was also reported by a study by Lao et al.[26] In the brain, GSH-Px but not catalase is active, thus, the antioxidant effect of curcuminoids in lowering GSH-Px in LHON patients in this study might have implications in the optic nerve tissue. The level of GSH-Px in healthy Thai subjects was 13.9 ± 1.05 kU/g Hb. Interestingly, the LHON patients with higher GSH-Px (higher than 14 kU/g Hb) seemed to respond better to curcuminoids as shown by their greater reduction in the GSH-Px than that of the patients with lower GSH-Px (data not shown). This suggested the effect of curcuminoids in normalization of the GSH-Px.

There might be some limitations in VA measurement in the study. In particular, the baseline VA of most of the patients in this study was quite severe (around finger count). The scale of improvement from hand motion to finger count to 20/200 was rather vague and rough. Therefore, in clinical practice, it would be rather difficult to accurately detect VA improvement from severely impaired VA baseline, if the magnitude of VA was unremarkably improved. Other limitations of the study included the inclusion of patients with longterm visual loss, patients with previously treated visual loss and the lack of follow-up beyond 48 weeks.

The administration of curcuminoids at the dose of 500 mg/day for 48 weeks in this study did not show clinically significant toxic effects as demonstrated by hematological profiles, renal function and liver function tests. The safety of curcumin has also been supported in other studies using the dose of 8,000 mg/day for up to 3 months or the high single dose of 12,000 mg.[25,26] The drop out rate in the curcumin group (18.5%) compared to the placebo group (9.1%) could affect the results of the toxicity of the treatment but we employed intention to treat analysis to minimize this bias. The adverse symptoms of the patients were minor and were distributed similarly in both curcumin and placebo groups.

In conclusion, with 500 mg/day of curcuminoids

for 48 weeks, we could not find significance effects of curcuminoids on the clinical parameters, visual electrophysiological parameters, and blood biochemical oxidative parameters in G11778A LHON patients as compared with the placebo. Nevertheless, we did find significant changes from baseline values of HVF-MD, and HVF-PSD in the curcuminoids group which may reflect subtle fenestrated scotoma visual filed improvement. The antioxidant effect of curcuminoids lowering GSH-Px in red blood cells was observed. Given the safety of curcuminoids, higher dose, longer duration, or larger trials would be likely to reveal more evident effects.

In addition, the role of curcumin as a neuroprotective agent has emerged and this might be of benefit in preventing visual loss in asymptomatic LHON mutation carriers or improving retinal ganglion cell function shortly after the onset of symptoms. Curcumin may be used as an adjunct to treatment of neurological disease with oxidative stress. A long-term prospective cohort of asymptomatic LHON mutation carriers will be needed to prove this role of curcuminoids.

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