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Clinical evaluation of the neuroprotective effect of α-tocopherol against glaucomatous damage

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PURPOSE. *To evaluate the vasoregulatory effect of antioxidant α-tocopherol on retina via protein kinase C pathway.*

METHODS. *Thirty glaucomatous patients (60 eyes) were included in this study. The patients were divided into three groups. For patients in Group A, tocopherol was not supplemented in their therapy. Patients in Groups B and C received 300 and 600 mg/day of oral α-tocopherol acetate, respectively. The ultimate blood tocopherol levels were confirmed via high-performance liquid chromatography assay. Progression of the disease for each subject was monitored via visual field measurements and color Doppler imaging of ophthalmic and posterior ciliary arteries at the beginning and at the 6th and 12th months of this study.*

RESULTS. *The average differences between the pulsatility indexes (PI) and resistivity indexes (RI) of both ophthalmic arteries (OA) and posterior ciliary arteries (PCA) of Groups B and C were significantly lower than those of Group A at months 6 and 12. In trial groups, RI decreases observed in PCAs at months 6 and 12 and PI decreases observed in OAs at the 6th month were statistically significant. Differences of mean deviations with visual fields in Groups B and C were highly significantly lower than that of Group A.*

CONCLUSIONS. *α-Tocopherol deserves attention beyond its antioxidant properties for protecting retina from glaucomatous damage. (Eur J Ophthalmol 2007; 17: 528-33)*

KEY WORDS. *Doppler, Glaucoma, Tocopherol, Vitamin E, Neuroprotection*

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INTRODUCTION

Tocopherols are known to be liposoluble antioxidant molecules prefixed as α, β, γ, or δ according to the methyl (CH_3) or proton (H) groups bound to their benzene rings, with RRR-α-tocopherol being the most biologically active form of them all (1). However, complex molecules that control tocopherol metabolism have recently been discovered, such as tocopherol transfer protein, α-tocopherol specific membrane receptors, and intracellular transfer proteins. The discovery of these delicate and intricate regulatory mechanisms aroused the idea that biological activity of this genre of molecules might be beyond their antioxidant function (2). Indeed, specific effects including protein kinase C (PKC) inhibition of α-tocopherol have been shown in many in vivo and in vitro studies which were not mimicked by antioxidants like Probucol and closed isomers like β-tocopherol (3).

As supplementation of vitamin E has been shown to be beneficial and protective for almost all of the eye tissues, now its antiproliferative effects (such as inhibition of Tenon capsule fibroblast proliferation, which is important for success in filtrating surgery) are in focus (4). PKC pathway also has further effects regarding glaucoma. PKC inhibitors relax trabecular meshwork (5) and effect matrix metalloproteinase and $PGF_{2\alpha}$ (6). Glutamate

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transporter activity has been shown to be modulated by PKC (7). But the most important effect pioneered in this study is the vasoregulatory effect of vitamin E and PKC in retina. In 1995, Kunisaki et al (8), and in 1999, Lee et al (9) showed in different models that retinal vascular dysfunction due to hyperglycemia could be prevented by α-tocopherol via diacylglycerol-PKC pathway. In this study, we clinically evaluated the preventative effect of α-tocopherol against vascular dysfunction due to glaucoma.

METHODS

Sixty medically controlled glaucomatous eyes with various etiologies of 30 patients were included in this study. While patients in Group A (n=9) were not receiving tocopherol, patients in Groups B (n=12) and C (n=9) were given 300 and 600 mg/day of oral RRR-α-tocopherol acetate, respectively. The mean ages, intraocular pressures (IOP), best-corrected visual acuities of 10/10 ratios, and disease etiologies of the patients that were recorded at the beginning of this study were well balanced (Tab. I).

IOP of each eye has been kept below 20 mmHg with medications that were not necessarily uniform. Differences between the subjects in terms of age, gender, additional systemic and ocular pathologies, C/D ratios, and number and variation of medications were not significant.

Color Doppler imaging (CDI) examinations were made with a Toshiba SSH 140-A and a 7.5 mHz linear transducer. Retinal blood flows of ophthalmic arteries (OA) and posterior ciliary arteries (PCA) were evaluated and resistivity indexes (RI) and pulsatility indexes (PI) were obtained at the beginning of the study, with follow-up examinations at the 6th and 12th months. The differences for individual resistivity (ΔRI) pulsatility indexes (ΔPI) were recorded at the 6th and 12th months for each patient. The data of Groups B and C were statistically compared with those of Group A. pAB and pAC values for 6th and 12th months were calculated.

Humphrey Instruments Inc. Field Analyzer Model 640 instrument was used for Fast pac visual fields. Mean deviation (MD) values at the beginning, 6th, and 12th months were recorded and mean deviation difference (ΔMD) values were calculated at the 6th and 12th months. ΔMDs of Groups B and C were statistically compared with those of Group A.

Final blood tocopherol levels of each patient were confirmed via high-performance liquid chromatography (HPLC) assay. The equipment used consisted of Waters 510 HPLC pump, Hypersil PEP 300 C18 (250×4.6 mm, 5 µm) colon, Waters 484 Tunable Absorbance UV Detector and Chromatography Station for Windows (CSW 1.7), and a Data System (Data Apex Ltd., Czech Republic, 1998). Samples were injected into 1 mL/min (50:40:10) methanol:acetonytrile:hexan mobile phase in room temperature and detected in λ_{max}:292 nm. Ultimate percentages of the peak areas were calculated.

For statistical analysis, Mann-Whitney *U*-test was used. Data were expressed as mean±standard deviation and $p<0.05$ was considered significant while $0.1>p>0.05$ was considered not quite significant.

RESULTS

Tocopherol uptake was confirmed with HPLC assay in Groups B and C with higher tocopherol peak areas (21.586±3.526% and 110.85±47.907%, respectively) than those of Group A (12.719±3.246%). Statistically, increase in tocopherol levels in Group B was not quite significant ($p=0.0754$), while that in Group C was significant ($p=0.0011$).

TABLE I - PATIENT DATA AT THE BEGINNING OF THE STUDY

	Group A	Group B	Group C
Number of patients	9	12	9
α-Tocopherol supplementation, mg/kg/d	N/A	300	600
Mean (SD) age, yr	58.4 (±6.4)	55.9 (±12.1)	57 (±6.3)
Mean (SD) intraocular pressure, mmHg	18.35 (±2.5)	20.62(±2.2)	20.95 (±3.9)
10/10 Best-corrected visual acuity, %	67	55.5	50
Etiology			
Secondary open angle glaucoma	5	7	4
Angle closure glaucoma	4	5	5

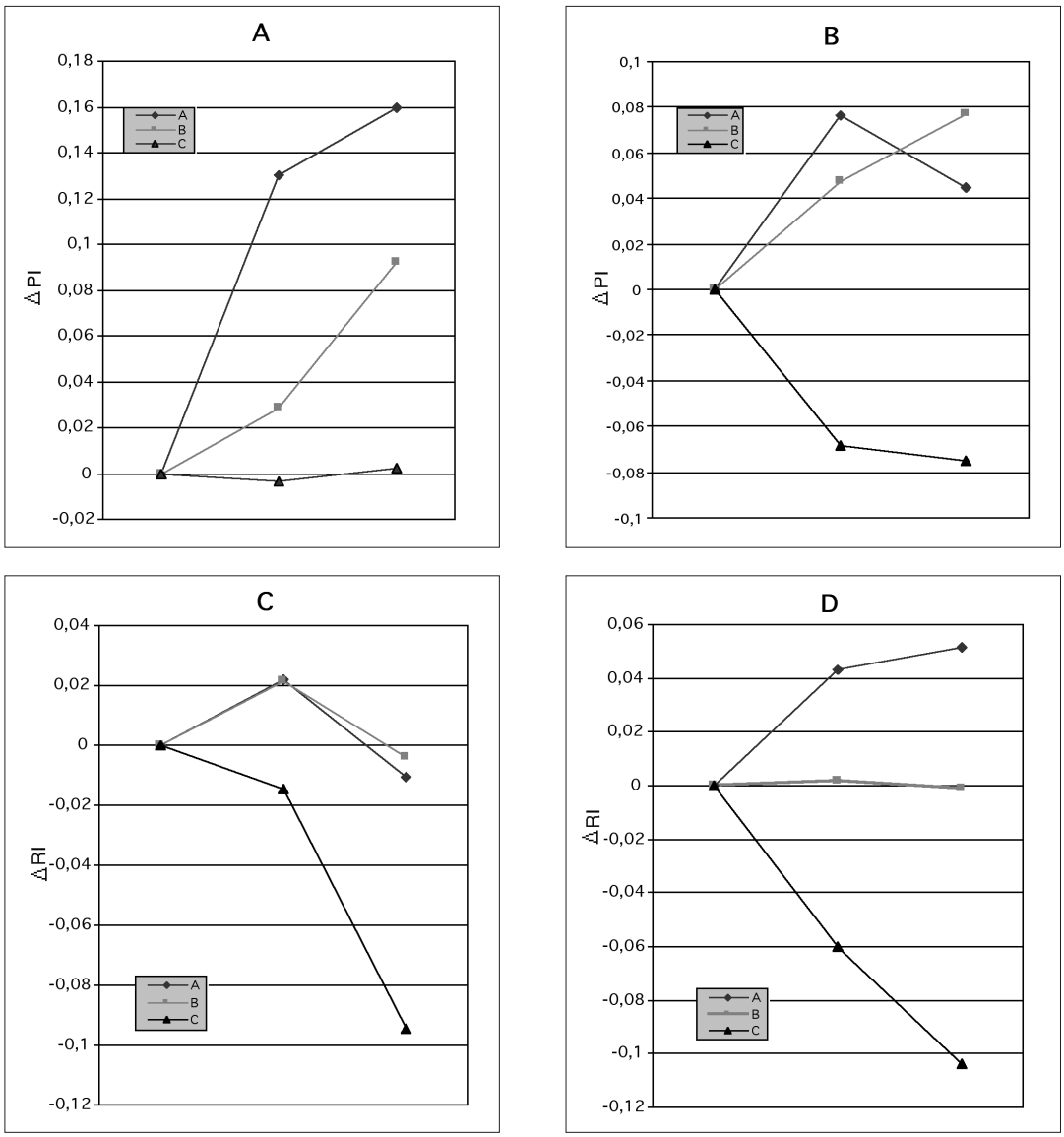

Fig. 1 - *Ophthalmic* **(A)** *and posterior ciliary* **(B)** *arteries mean ΔPI, ophthalmic* **(C)** *and posterior ciliary* **(D)** *arteries mean ΔRI values at 6 and 12 months.*

ΔPIs and ΔRIs of OAs and PCAs in 6th and 12th months are displayed in Table II. There was no statistically significant evidence to suggest that these results are influenced by gender differences.

When compared with Group A, trial groups displayed time- and dose-dependent improvements in all parameters (Fig. 1). In OAs, only statistically significant decrease was found in the 6th month ΔPIs of Group B. However, in Group C, decreases observed in ΔRIs at the 6th month visit and those in ΔPIs in both 6th and 12th month visits were not quite significant. In PCAs, ΔRIs in both 6th and 12th month visits were statistically significant (Tab. III).

Compared with those in Groups B and C, Group A patients in displayed a statistically significant reduction in ΔMD values in 6th and 12th months (Fig. 2, Tab. IV, Tab. V).

DISCUSSION

The vital role vitamin E plays in all tissues, including the eye, is its antioxidant effect. Along with all other disciplines, research findings in ophthalmology – and glaucoma – were attributed to this effect. But since 1989, effects of vitamin E beyond that were gradually discovered. Another distinguishing feature of vitamin E from other antioxidants is that there are delicate mechanisms adjusting its

tissue levels. In other words, organisms exert a certain effort to maintain vitamin E concentration in appropriate levels (3), and we have specific evidence suggesting that the tissues within the eye are not exceptions (10). Therefore, during both planning and interpretation of this study, possible effects of vitamin E beyond its antioxidant nature have been taken into consideration.

Tocopherol levels have been assayed with HPLC. Nevertheless, peaks have not been compared with a standard. That may constitute a weakness for the study in the means of reliability of the obtained tocopherol levels. On the other hand, results are sufficient to confirm increased tocopherol uptake in Groups B and C.

Whether the given tocopherol actually increases the retinal vitamin E levels or not can be the topic of another discussion. It is known that vitamin E accumulates within the retina when administered via oral or parenteral route (11). It also has been shown that daily doses of 300-600 mg/kg/day are low, and much higher doses can safely be given to the patients (1). According to our literature scan,

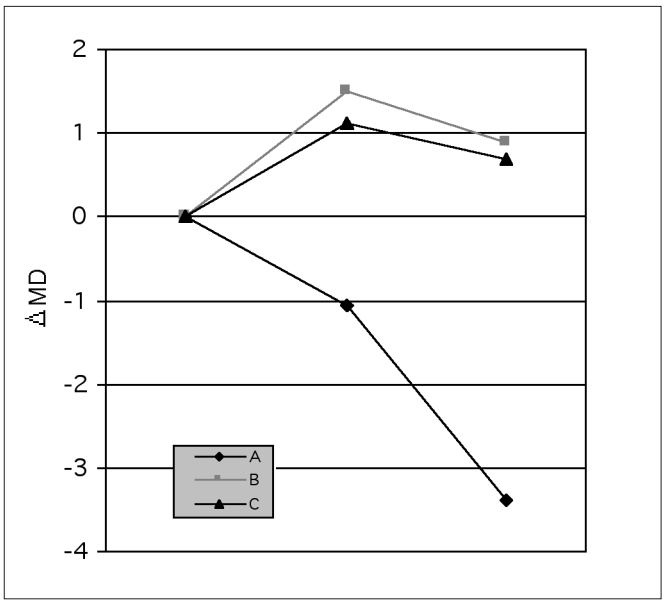

Fig. 2 - *Variations of mean ΔMD values at 6th and 12th months in the groups compared with the beginning of the study.*

TABLE II - DIFFERENCE OF PULSATILTY INDEXES (ΔPI) AND DIFFERENCE OF RESISTIVITY INDEXES (ΔRI) IN 6th AND 12th MONTHS OF OA AND PCA

	A (6 mo)	B (6 mo)	C (6 mo)	A (12 mo)	B (12 mo)	C (12 mo)
OA						
ΔPI	0.126±0.19	0.027±0.19	–0.003±0.27	0.159±0.34	0.092±0.29	0.002±0.39
ΔRI	0.022±0.08	0.022±0.11	–0.014±0.15	–0.010±0.12	–0.04±0.14	–0.094±0.28
PCA						
ΔPI	0.761±0.31	0.475±0.23	–0.672±0.29	0.044±0.33	0.077±0.43	–0.075±0.36
ΔRI	0.043±0.12	0.002±0.08	–0.060±0.14	0.052±0.21	–0.001±0.17	–0.104±0.22

OA = Ophthalmic arteries; PCA = Posterior ciliary arteries

TABLE III - STATISTICAL ANALYSIS OF PULSATILTY INDEXES (ΔPI) AND RESISTIVITY INDEXES (ΔRI) VALUES OF OA AND PCA IN 6th AND 12th MONTHS

	p A, B (6 mo)	p A, C (6 mo)	p A, B (12 mo)	p A, C (12 mo)
OAΔPI	0.0275*	0.0706†	0.1703‡	0.0920†
OAΔRI	0.3236‡	0.0605†	0.4344‡	0.1878‡
PCAΔPI	0.4696‡	0.1028‡	0.3997‡	0.1595‡
PCAΔRI	0.1767‡	0.0275*	0.2229‡	0.0249*

*p<0.05, significant; †0.1>p>0.05, not quite significant; ‡p>0.1, not significant.
OA = Ophthalmic arteries; PCA = Posterior ciliary arteries

TABLE IV - DIFFERENCE OF MEAN DEVIATIONS (ΔMD) AT 6th AND 12th MONTHS

	6th mo	12th mo
A, ΔMD	–1.046 ± 3.35	–3.386 ± 6.7
B, ΔMD	1.504 ± 2.41	0.880 ± 3,31
C, ΔMD	1.107 ± 2,13	0,686 ± 2,01

TABLE V - STATISTICAL ANALYSIS OF ΔMD VALUES AT 6th AND 12th MONTHS

p A, B (6 mo)	0.0009*
p A, C (6 mo)	0.0299†
p A, B (12 mo)	0.0003*
p A, C (12 mo)	0.0026†

*p<0.01, very significant; †p<0.05, significant.

there are no studies about the effects of tocopherol in management of glaucoma.

One of the mainstay modalities in evaluating retinal blood flow – a reliable method – retinal CDI (12) has been used in this study and findings of the OAs and PCAs have been evaluated. Due to the tortuous course of PCAs within the optical nerve, it may not always be easy to obtain a validly accurate measurement (13). Nevertheless, PCAs had been studied, for since it supplies the beginning of the optical nerve, it influences the pathogenesis and prognosis of glaucoma (12). As far as PCA is concerned, regardless of CDI findings, RI is assumed to be the most accountable measurement parameter (14). The RIs of the PCAs are reported to be increased in glaucomatous patients due to various etiologies (15), which were lower in glaucomatous patients following trabeculectomy (13).

The study was carried out on glaucomatous patients whose IOPs were kept under control via medical treatment. At this point, various medical therapy regimens may produce unpredictable effects on the efficacy of any drug administered (16, 17). Therefore, while planning the study, a predomination of a given therapy regimen within a particular group has been avoided.

Consequently, ΔRI and ΔPI values – except OA ΔRI – were found to be increased in Group A, with Groups B and C demonstrating lower levels. This difference was observed to show a dose – time related characteristic.

All the basic and increased RI and PI findings obtained in our study are consistent with the findings in the literature (13, 18). Compared with Group A, all the ΔRI and ΔPI values were found to be decreased at this study. Of these, PCA ΔRI values in 6th and 12th months and OA ΔPI values in the 6th month were statistically significant. PCA has a lower resistance than OA and expressivity of RI is higher for low resistance arteries, whereas for high resistance arteries, PI is more expressive (14, 19).

ΔMD is a reliable parameter for following up visual field integrity (20). Results clearly indicate the preventative effect of α-tocopherol from the visual field loss which Group A subjects seem to experience. Decrease and elimination of visual field defects and visual field loss by administration of vitamin E combined with vitamin B complex and Cosaldon A have been shown in glaucomatous patients (21, 22). However, the dramatic improvement observed in our study can be partially attributed to the use of α-tocopherol instead of synthetic preperates which were consisted of equal amounts of eight isomers (23).

As a result of the studies on its pathogenesis, glaucoma has been renamed optic neuropathy, with increase in IOP assumed as the major risk factor. This outcome has led to a tendency to the use of neuroprotective drugs, and vitamin E has been used likewise for some time.

In this study, the vasoregulator and neuroprotective effects of α-tocopherol – a synthetic, safe, and economic drug – in glaucomatous patients have been clinically demonstrated. The dosage used in this study is known to be insufficient to produce a significant antioxidant effect. This was arranged in order to accentuate the possible effects of this agent over the PKC pathway (which it inhibits). The significant improvements we have achieved despite the low dosage we have used is highly attributable to this property of α-tocopherol.

Our findings suggest that, in the pathogenesis and treatment of glaucoma, α-tocopherol merits a concern beyond its antioxidant effect.

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