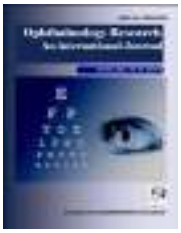

Ophthalmology Research: An International Journal
2(1): 1-9, 2014, Article no. OR.2014.001

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Effects of Micronutrients on Serum Antioxidant Status of Glaucoma Patients: A Randomized, Placebo-controlled, Double-masked Pilot Study

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Authors' contributions

This work was carried out in collaboration between all authors. Authors ALY, OM, CE, UWL designed the study. Author OM conducted the research. Authors ALY, CE and UWL analyzed the data and wrote the manuscript. All authors read and approved the final manuscript.

Original Research Article

Received 9th July 2013
Accepted 22nd September 2013
Published 15th October 2013

ABSTRACT

Aims: Glaucoma is a chronic eye disease, which is associated with progressive optic neurodegeneration and concomitant visual field defects. Besides an elevated intraocular pressure, recent studies have suggested that other risk factors such as oxidative stress may play an important role in the pathogenesis of glaucoma. The goal of this study was to examine the effects of oral micronutrients on the antioxidant status in glaucoma patients.
Study Design: randomized, placebo-controlled, double-masked pilot study.
Place and Duration of Study: Ophthalmological Clinic Oculus Parter-Bucharest, Romania, between March 2007 and February 2008.
Methodology: This study was conducted with 40 glaucoma patients receiving either oral supplementation of micronutrients containing 150 mg α-lipoic acid, 36 mg vitamin E, 70 mg vitamin C, 3.6 mg vitamin B1, 5 µg vitamin B12, and 100 mg bilberry extract (Ocuvite® Glaukom) or placebo tablets for a duration of six months. Effects of oral micronutrients on uric acid, ascorbic acid, and tocopherol serum concentrations were investigated in a subgroup of 25 patients. The safety of the supplementation was assessed in 40 patients.
Results: Supplementation with oral micronutrients for six months showed a minor

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increase of antioxidant serum levels without statistical significance (uric acid: $P = 0.14$; ascorbic acid: $P = 0.11$; tocopherol: $P = 0.32$). Safety data were satisfactory.
Conclusion: There were no statistically significant differences between both treatment groups, which may be explained by the short study period and the small sample size. Further extensive studies are required to verify the hypothesis that oral supplementation of micronutrients may influence the antioxidant status in glaucoma patients.

Keywords: Micronutrition; antioxidant; glaucoma; oxidative stress; supplementation.

ABBREVIATIONS

BMI: Body Mass Index; C:D: cup-to-disc-ratio; HPLC: High performance liquid chromatography; IOP: intraocular pressure; LA: α-lipoic acid; POAG: primary open-angle glaucoma; ROS: reactive oxygen species; VA: visual acuity.

1. INTRODUCTION

Glaucoma is a chronic eye disease, which is associated with a progressive degeneration of the optic nerve. Currently, the primary therapy to slow down the progression of glaucoma is to decrease the intraocular pressure by either eye drops or surgical intervention. In recent years, there is a growing body of evidence showing that not only the elevated intraocular pressure or genetic factors [1], but also oxidative stress may affect and promote glaucomatous optic neurodegeneration [2-5]. In general, oxidative stress is defined as a resultant of reactive oxygen species (ROS) overproduction as compared to its antioxidative capacity [6]. To limit oxidative injury in a cellular system, it is therefore important to decrease ROS production or to increase its antioxidative capacity. In ocular diseases, micronutrients with antioxidative effects are increasingly discussed as therapeutic options for glaucoma [7]. Several studies have found decreased levels of antioxidants such as glutathione, vitamin C and E in the serum of glaucoma patients as compared to control patients [8,9]. Similarly, the antioxidant status was also reduced in the aqueous humor of glaucoma patients [10,11]. However, it is still unclear whether or not supplementation with micronutrients may improve the pro-oxidant-antioxidant balance in patients with glaucoma.

This study was conducted to assess the effects of micronutrients, as presented in the nutritional formula Ocuvite® Glaukom (Dr. Mann Pharma, Bausch & Lomb, Berlin, Germany), on markers of the antioxidant status in the serum of glaucoma patients. The daily dose of Ocuvite® Glaukom contains 150 mg α-lipoic acid, 36 mg vitamin E, 70 mg vitamin C, 3.6 mg vitamin B1, 5 µg vitamin B12, and 100 mg bilberry extract. These results may provide further insights into the importance of the balance between oxidative stress and the antioxidant defence system in glaucoma patients.

2. MATERIALS AND METHODS

2.1 Study Design of the Pilot Clinical Trial

In this randomized, placebo-controlled, double-masked pilot study, a total of 40 patients suffering from primary open-angle glaucoma (POAG) were enrolled in the Ophthalmological Clinic Oculus Parter-Bucharest, Romania. The inclusion and exclusion criteria for participation in this study were listed in Table 1 and Table 2. All patients gave written

informed consent. The study was approved by the Ethics Committee Freiburger Ethik-Kommission International, University of Freiburg, Germany, and followed the Tenets of the Declaration of Helsinki for research involving human subjects.

Table 1. Inclusion criteria for patients

Inclusion criteria
1. Adult (men or women) suffering from primary open-angle glaucoma with intraocular pressure <20 mm Hg (treated with local therapy). Primary open-angle glaucoma was defined as optic disc changes with a cup:disc ratio (C:D) > 0.5 and visual field deficits of at least the parameters of an automated perimeter (Mean Deficit, Pattern Standard Deviation) outside the normal range
2. Age between 40 and 60 years
3. Intraocular pressure controlled only with local antiglaucomatous medication
4. Visual acuity of at least 0.5 (decimal fractions)

Table 2. Exclusion criteria for patients

Exclusion criteria
1. Taking any concomitant medication
2. Vasospastic syndrome (migraine, Raynaud)
3. Coronary heart disease
4. Stroke
5. Obesity (Body Mass Index>30)
6. Cardiovascular risk factors: Hypertension (systolic ≥140, diastolic ≥ 90 mm Hg), known hyperlipidemia, diabetes mellitus, smokers (> 3 cigarettes/day)
7. Severe systemic or ocular disease
8. Autoimmune disease
9. Secondary glaucoma and angle closure glaucoma
10. Exfoliation syndrome
11. Other ocular degenerative disease (cataract, diabetic retinopathy, Age-Related Macular Degeneration)
12. History of eye surgery (including laser), trauma
13. Drug, alcohol abuse
14. Known sensitivity to the tested ingredients
15. Supplementation with antioxidants within the previous three months
16. Any concomitant nutritional supplementation
17. Involvement in the last 30 days in any other investigational drug study
18. Pregnant and lactating women
19. For whom, in the physician's opinion, any of the protocol procedures posed a special risk not outweighed by the potential benefits of participating in the study
20. Who were unlikely to comply with the study protocol or who were likely to be moving and lost to follow-up in the study period
21. Who planned to start a diet or to change their diet during the course of the study

2.1.1 Micronutritional supplementation

Patients were randomized to receive tablets from either the micronutritional supplementation (Ocuvite® Glaukom, Dr. Mann Pharma, Bausch & Lomb, Berlin, Germany) or placebo. Two micronutritional formulation tablets (daily dose) of Ocuvite® Glaukom contained the nutritional ingredients: 150 mg α-lipoic acid (LA), 36 mg vitamin E (α-tocopherol), 70 mg

vitamin C (calcium ascorbate), 3.6 mg vitamin B1 (thiamin), 5 µg vitamin B12 (cyanocobalamine), and 100 mg bilberry extract (containing at least 25 % anthocyane). Each patient was required to take one tablet twice a day orally. The control group received placebo tablets consisting of lactose, cellulose, and magnesium stearate. Packaging and labelling of the investigational products were done according to the labelling requirements of the EU Directive 2001/20/EC and all local requirements. The duration of supplementation was six months. A baseline visit was performed at Day 0, follow-up phone calls were placed at Day 30 and Day 100 and the final visit took place at Day 180 (±1 week). Blood samples were taken at Day 0 to obtain baseline values and at Day 180 for the acquisition of follow-up values. Patients' compliance was monitored by tablet count at the end of the supplementation period. Two patients in the placebo group discontinued treatment without completing the study (one was lost to follow-up and one had an adverse event). Since 13 of the blood samples (micronutrients n = 7, placebo n = 6) were thawed due to an electricity outage, the following blood sample analyses were conducted on the results of eventually 25 patients (micronutrients n = 13, placebo n = 12).

2.2 Serum Sample Collection and Analysis

Efficacy variables in the study were the serum status of uric acid, ascorbic acid, and tocopherol. For the analyses, blood samples were obtained from each patient at Day 0 and Day 180. The blood samples were drawn from the antecubital vein and the samples were processed and centrifuged at 2000 g for 10 minutes. After centrifugation, the supernatants were transferred with a pipette to Eppendorf tubes (4 x 1 ml) and frozen at -70℃. All serum samples were sent within 24 hours to Professor W. M. Nau, Jacobs University, Bremen, Germany, for the measurements of the antioxidant parameters.

High performance liquid chromatography (HPLC) was used to determine the concentrations of uric acid, ascorbic acid and tocopherol in serum samples. HPLC was performed using Polaris columns (Ether-RP-18-A-250/4.6/5). Elutants used were 20 mmol/l KH_2PO_4; 3.9 mmol/l phosphoric acid; 5% acetonitrile for uric and ascorbic acid measurements and 75% acetonitrile/ 25% methanol for tocopherol measurements. For uric acid and ascorbic acid analyses, a 200 µl aliquot of serum was mixed with 200 µl of orthophoric acid and perchloric acid (mixture ratio 6:3), thereafter 20 mmol/l EDTA solution for oxidation protection was added. For tocopherol measurements, a 100 µl sample was diluted with 400 µl ethanol to precipitate the proteins. All samples were centrifuged at 12000 rpm in an Eppendorf table centrifuge for 6 minutes. Each HPLC measurement was run with 10 µl of supernatants, and then repeated with 20 µl for the lower concentrations. The chromatographic run was monitored at an absorbance detection of 265 nm (for uric acid and ascorbic acid) and 280 nm (for tocopherol) and at a flow rate of 1.0 ml/minutes at 25℃.

2.3 Safety Variables

Safety was assessed by means of the adverse event report and the ophthalmic examination including a slit lamp examination, visual acuity testing, intraocular pressure measurement, funduscopy, and visual field analysis. The safety population included 40 patients.

2.4 Statistical Analysis

Statistical analyses were carried out with SAS® software version 9.1 or higher. Descriptive statistics were used for the calculation of mean values and standard deviation. For the

comparison of the two treatment groups, a Student's t-test or a Mann-Whitney test was used, depending on data normal distribution or not. Statistical significance was set as $P < 0.05$.

3. RESULTS

3.1 Demographics and other Baseline Characteristics

Demographics were generally similar between treatment groups. The mean age of the patients in the micronutrient supplemented group was 49.8 +/- 6.6 years and in the placebo group 52.2 +/- 6.7 years. 53.8 % (7/13) of patients in the micronutrient supplemented group and 75 % (9/12) in the placebo group were women. Body Mass Index (BMI) was very similar in both treatment groups (micronutrient supplemented group: mean BMI = 25.7 +/- 2.4 kg/m^2 versus placebo group: mean BMI = 25.1 +/- 2.3 kg/m^2). The mean systolic to diastolic blood pressure was 121.5 +/- 12.8 mmHg to 73.1 +/- 7.2 mmHg in the micronutrient supplemented group and 119.2 +/- 13.8 mmHg to 72.9 +/- 8.4 mmHg in the placebo group. All patients had primary open-angle glaucoma (POAG), as required for study entry. The baseline best-corrected visual acuity (VA) (decimal fractions), intraocular pressure (IOP), cup-to-disc-ratio (C:D) and visual field mean deficiency were similar between treatment groups. Baseline values of uric acid, ascorbic acid, and tocopherol were within normal ranges and similar between treatment groups.

3.2 Uric Acid Status in Serum

The increase of serum uric acid level from baseline to six months was higher for the micronutrient supplemented group [mean change: 65.5 µmol/L (increase of 25.9 %)] than for the placebo group [mean change 30.2 µmol/L (increase of 11.7 %)] (Table 3). However, there was no significant difference between treatment groups ($P = 0.14$).

3.3 Ascorbic Acid Status in Serum

Serum ascorbic acid slightly increased from baseline to six months in micronutrient-treated patients [mean change: 3.8 µmol/L (increase of 10.6 %)] and declined in placebo-treated patients [mean change: -10.3 µmol/L (decrease of 30.1 %)] (Table 3). Difference between both treatment groups was not significant ($P = 0.11$).

3.4 Tocopherol Status in Serum

There was a slight increase from baseline to six months in the serum tocopherol status of both treatment groups (Table 3). The increase in serum tocopherol status of micronutrient supplemented group [mean change: 4.6 µmol/L (increase of 15.2 %)] was insignificantly higher than of the placebo group [mean change: 1.2 µmol/L (increase of 4.1 %)] ($P = 0.32$).

Table 3. Serum antioxidant levels

	Ocuvite® Glaukom (n = 13)	Placebo (n = 12)	*P*-value#
Serum uric acid (µmol/L)*			
Baseline	252.7 +/- 62.0	258.3 +/- 43.6	
6 months	318.2 +/- 91.6	288.4 +/- 52.1	
Change from baseline	65.5 +/- 60.1	30.2 +/- 56.5	0.14
Serum ascorbic acid (µmol/L)*			
Baseline			
6 months	35.8 +/- 23.7	34.2 +/- 17.8	
Change from baseline	39.6 +/- 19.3	23.9 +/- 17.0	
	3.8 +/- 14.4	-10.3 +/- 26.1	0.11
Serum tocopherol (µmol/L)*			
Baseline	30.3 +/- 10.6	29.5 +/- 10.4	
6 months	34.9 +/- 9.0	30.7 +/- 7.2	
Change from baseline	4.6 +/- 7.0	1.2 +/- 9.9	0.32

** Serum concentrations as mean ± s.d.*

 # P-values from Student's t-test or Mann-Whitney depending on data distribution normality.

3.5 Safety Endpoints

The lids, conjunctiva, cornea, iris, and anterior chamber were examined at baseline and at six months. There were no abnormal safety findings on any of the slit lamp findings in the eyes of both treatment groups. There were also no significant changes in the best-corrected VA, IOP, and visual field mean deficiency values from baseline to six months**.** As evaluated with funduscopy, there were no abnormal changes in the macula, optic nerve head, or C:D in the eyes of both treatment groups. No serious ocular adverse events were recorded during the study. There were two reports of serious non-ocular adverse events. Both were considered to be unrelated to study treatment and occurred in the placebo group. One patient reported an acute allergic bronchitis. Another patient had bilateral breast carcinoma.

4. CONCLUSION

Antioxidants have been more and more discussed as an adjuvant therapy in various oxidative stress-associated diseases [12-14]. There are an increasing number of studies showing that oxidative stress may also be involved in the pathogenesis of glaucoma [2-5]. The goal of this study was to evaluate whether or not oral antioxidants have protective effects in glaucoma patients. We conducted a randomized, double-masked, placebo-controlled pilot study including 40 primary open-angle glaucoma (POAG) patients, who were administered oral supplementation with micronutrients (Ocuvite® Glaukom) versus placebo tablets for six months. Ocuvite® Glaukom is an oral micronutritional supplementation which consists of a combination of α-lipoic acid (LA), vitamins C, E, B1, B12, and bilberry extract. LA is a natural, potent thiol antioxidant which can also regenerate a number of physiological antioxidants of the lipid and aqueous phase [15]. Vitamin C is the most important hydrophilic antioxidant. Previous studies have found lower levels of vitamin C in the serum and aqueous humour of glaucoma patients [16,17]. Vitamin E is the most important lipophilic antioxidant and thus protects cell membranes from oxidation [6]. LA, vitamin C, and vitamin E are reported to have synergistic antioxidant effects [6,18]. LA and vitamin B12 are supposed to have neuroprotective properties, and vitamin B1 is involved in nervous system's conduction

[19-21]. Lower levels of vitamin B1 have already been detected in the serum of glaucoma patients [19]. Bilberry contains anthocyanins with antioxidant activity and neuroprotective effects against retinal neuronal damage [22].

In this preliminary study, we used a treatment time period of six months, which was regarded as suitable as compared to previous *in vivo* studies on oral antioxidant supplementations [23,24]. To exclude concomitant factors, the selected patients did not use any treatment other than antiglaucomatous eye drops, since some systemic antihypertensive agents and hypolipidemic drugs were able to decrease oxidative stress [25,26]. Furthermore, the patients in both treatment groups were adjusted for their body mass index, their intraocular pressure level and their degree of glaucomatous optic degeneration. The body mass index is a common parameter to assess nutritional status and is essential for the comparison of both groups, since the intake of food may influence the antioxidant levels in the serum [27].

Our results demonstrated that oral intake of micronutrients such as Ocuvite® Glaukom for six months is a safe administration in glaucoma patients. Visual acuity, intraocular pressure, cup-to-disc-ratio, and visual field parameters were generally stable in both treatment groups. Furthermore, no serious ocular or non-ocular adverse events occurred in the micronutrient supplemented group. The mean serum concentration of uric acid, ascorbic acid and tocopherol showed a slightly higher increase in the micronutrient supplemented group than in the placebo group after six months of supplementation. However, the differences between both treatment groups were not statistically significant, which may be explained by the high data variations and the small sample size. Another reason may be that clinical significance of antioxidant supplementation will only be detected after a long-term period of intake or that there are too many, partly unknown, concomitant factors, which may influence the cellular redox system. We have chosen the antioxidants uric acid, ascorbic acid and tocopherol, since they were regarded as important contributors to the serum antioxidant activity [28]. In previous studies, it has been shown that oral supplementation with micronutrients could improve antioxidant status in human immunodeficiency virus-infected patients [23] and decrease serum levels of oxidative stress markers in healthy adults [24].

This is a first pilot study evaluating the effects of oral supplementation with micronutrients on the antioxidant status of glaucoma patients. Our results have demonstrated that oral supplementation with micronutrients for six months is a safe administration in glaucoma patients. Further extensive studies are required to verify these results and to demonstrate a clearer differentiation from placebo, especially on a larger population and with more specific and sensitive markers for the antioxidant status.

CONSENT

All authors declare that written informed consent was obtained from the patients for including their data into this study.

ETHICAL APPROVAL

All authors hereby declare that all experiments have been examined and approved by the appropriate ethics committee and have therefore been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki.

ACKNOWLEDGEMENTS

The authors thank Professor W. M. Nau and his team, School of Engineering and Science, Jacobs University, Bremen, Germany, for quantification of the antioxidant serum levels. This research was funded by Bausch & Lomb, Dr. Mann Pharma GmbH, Berlin, Germany. There was no involvement of the study sponsor in the interpretation of the data.

COMPETING INTERESTS

There is commercial relationship by research funding by Bausch & Lomb, Dr. Mann Pharma GmbH, Berlin, Germany.

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Peer-review history:
The peer review history for this paper can be accessed here:
http://www.sciencedomain.org/review-history.php?iid=297&id=23&aid=2257