The effect of Ubiquinone administration on oxidative DNA damage and repair in plasma levels in non-proliferative diabetic retinopathy

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ABSTRACT

Background: Diabetic retinopathy (DR) initially is denominate non-proliferative DR (NPDR), and has the possibility to develop to proliferative DR (PDR). Aim: To determine the effect of Ubiquinone on the oxidative DNA damage and DNA repair in plasma levels in NPDR. Methods: Through a double-blinded, placebo-controlled, clinical trial, 20 patients were treated with Placebo and 20 patients with 400 mg/day of Ubiquinone (Coenzime Q10) for 6 months. Inclusion criteria were male or female, with type 2 diabetes mellitus and NPDR. The informed consent was signed. The DNA oxidative damage was determinate by the plasma levels of 8-hydroxy-2'-deoxyguanosine (8-OHdG) and DNA repair by the 8-oxoguanine-DNA-N-glycosylase-1 (hOGG1). Superoxide Dismutase (SOD) activity and CoQ10 were assessed as antioxidants. Results: Levels of the 8-OHdG were significantly increased in baseline measurements in Ubiquinone and Placebo group, with significant decrease (p=0.05) in the Ubiquinone group at the end of the study. Levels of DNA repair enzyme hOGG1 were increased in baseline 0.42 ± 0.03 ng/mL–final results 0.43 ± 0.05 ng/mL in Ubiquinone group. The SOD activity were diminished in Ubiquinone and Placebo groups at baseline (p=0.006, p=0.01 respectively), even in Placebo group, the SOD decreased even more (p=0.02). The endogenous antioxidant (CoQ10) was consumed at baseline in both groups and improved to normal levels at the end only in the Ubiquinone group (p<0.001). Conclusion: We observed an increase of 8-OHdG in the baseline determination to patients with NPDR suggesting DNA damage with the repairing enzyme trying to compensate for oxidative damage. The CoQ10 supplementation could diminish the oxidative DNA damage in NPDR.

Introduction

Diabetic retinopathy (DR) is one of the most common micro vascular complication in diabetes mellitus (DM) that progresses from non-proliferative diabetic retinopathy (NPDR) to proliferative diabetic retinopathy (PDR) [1-2]. Chronic hyperglycemia is directly related to the development and progression of DR [3], by activation of the biochemical mechanisms that directly participate in the development of micro vascular damage through: a) the accumulation of sorbitol, b) the formation of advanced glycation end-products (AGEs), c) activation of the protein kinase C, and, d) the appearance of inflammation and oxidative stress [4]. Inflammation, oxidative stress, hyperglycemia, and redox homeostasis are events that participate in the development of DR [4]. In normal cellular metabolism few reactive oxygen species (ROS) are produced; however, in the hyperglycemic state the production of ROS is increased. During oxidative phosphorylation of the mitochondria there is an electron transfer to oxygen, which

results in formation of the superoxide anion (O_2^-) that converts into hydrogen peroxide by the superoxide dismutase (SOD) or transforms into the hydroxyl radical. The O_2^- is a highly reactive species capable of reacting with proteins, lipids, and nucleic acids [5]. The 8-hydroxy-2'-deoxyguanosine (8-OHdG) is a sensitive and specific marker of oxidative damage to genetic material with important mutagenic potential [6,7]. In human cells the oxidative damage is primarily repaired by the endonuclease enzyme 8-oxoguanine-DNA-N-glycosylase-1 (hOGG1) through mechanisms of base excision [8].

The Ubiquinone or co-enzyme Q10 (CoQ10) is an important nutrient that acts on the chain of electron transport in mitochondria and the synthesis of ATP [9].

Ubiquinone is a potent antioxidant that provides cells with protection against oxidative damage [10,11]. Previous studies have shown that patients with DM are deficient in CoQ10 endogenous levels [12]. Experimental and clinical reports have described the protective effect of CoQ10 supplementation on DM complications such as diabetic nephropathy and DR [13,14]. To date, there is no evidence on the effect of CoQ10 administration on oxidative DNA damage and DNA repair in patients with NPDR. The objective of the study was to determine the effect of Ubiquinone on the oxidative DNA damage and DNA repair in plasma levels in NPDR.

Materials and methods

Study design, and study population

A randomized, double-blind placebo-controlled clinical trial was performed in patients with type 2 DM with NPDR without macular edema. We made a parallel assignment of subjects by means of a computer-based blocking method of randomization and a 1:1 allocation. Patients with age ranging from 30-75 years old were included, with glycated hemoglobin (HbA1c) <12%, without ocular pathologies that could affect or interact with the results; and without intake of antioxidants during 6 months previous.

Study procedure

Forty patients in the study were enrollment. The patients were instructed to take one daily dose of Placebo or CoQ10, 400 mg. We also included clinically healthy individuals of similar age, gender and weights, to establish normal values of the reagents. We performed randomization through a computer-based code assignation. A pharmaceutical packed the medication in vials containing 30 units each, with similar appearance, size and form. One of the authors was in charge of delivering the vials every 4 weeks, previously tagged with the assigned codification. We were in constant communication with the patient´s physician to ensure they followed their usual medication. The patients and their doctors were kept blinded to avoid bias. To guarantee the medication adherence we gave each patient a diary where they had to record the time and date they took the medication, and describe the adverse events during the follow-up. Every month the diary was revised and pill count was performed by another co-researcher to grant adherence and medication safety. We also requested new liver and kidney function tests, and lipid profiles each month to ensure the drugs were safe. At each clinical visit other research counted the medications to quantify the drug ingested in each group monthly. He considered 80% ingestion of the medications as minimum compliance, and dispensed the medications for the following month throughout the study period. We evaluated the correctness of the data registration in a case report format. Patients were followed by their family physicians who managed the DM and their diets.

In this study, the antioxidant Ubiquinone was as adjuvant therapy in combination with hypoglycemic, antihypertensive, and hypolipidemic drugs. In addition, the family doctors in clinic followed medical recommendations for nutritional therapy in the management of adults with DM, as well as the physical activity considerations for diabetic complications; both of which were based on the American Diabetes Association (ADA) guidelines [15]. Fasting glucose, HbA1c (To monitor metabolic control).

The sample size was calculated based on Superoxide dismutase (SOD) activity, taking into account a difference of 3.5 IU/mL for the interventional groups, with a 95% level of significance, and a statistical power $(1-\beta)$ of 80%. The randomization was done by an external researcher. There were 20 patients obtained per group.

Analysis of markers

Five mL of venous blood was obtained in a tube containing Ethylenediaminetetraacetic acid (EDTA). The plasma were extracted at 1800 revolutions per minute (rpm) × 10 min, and

stored at -80°C until final processing. All of the technical readings of optical density were made with the Synergy HT (BIOTEK) microplate reader.

■ 8-hydroxy-2'-deoxyguanosine

Instructions for the ELISA kit were followed (8-hydroxy-2-deoxyguanosine No. ab10124 Abcam°, Cambridge, United Kingdom). The plasma sample, the EIA buffer, the standards, and the 8-OHdG-AChE tracer were added to all the wells except the blank. The monoclonal antibody 8-OHdG was added, and the plate was incubated for 18 h at 4°C and washed with buffer for the recommended times, and 200 μL of Ellman's reactive was added to each well. The optical density was read at 405 nm.

■ 8-oxoguanine-DNA-N-glycosylase-1

The kit manufacturer's instructions (Human-8-oxoguanine-DNA-glycosylase MBS702793 MyBiosource°, San Diego, CA, USA) were followed. The reagents and samples were prepared for the indicated dilutions. 100 μL of plasma and standards were added to the wells and the plate was incubated at 37°C. The biotinylated antibody was added and incubated under the same conditions. The corresponding washings were done and the HRP-avidin was added, followed by the substrate, and then the stop solution at the corresponding times. The optical density was read at 450 nm.

■ Superoxide dismutase

The kit manufacturer's instructions were followed (SOD No. 706002, Cayman Chemical Company°, USA). The detection of O2 generated by the xanthine oxidase and hypoxanthine enzymes was through the reaction of tetrazolium salts. The plasma samples were diluted 1:5 in sample buffer: 200 μL of the radicals' detector, diluted 1:400, was placed, and 10 μL of the sample was then added. After slow agitation, 20 μL of xanthine oxidase was added to the wells. The microplate was incubated 20 min at room temperature and the optical density was read at a wavelength of 440 nm.

■ Coenzyme Q10

Plasma concentrations were obtained following the kit manufacturer's instructions (Human coenzyme Q10 No. CSB-E14081h, Cusabio° Biotech Co., Ltd, China). The sample was diluted 1:100 and 50 μL of the sample or standard was placed in each well and 50 μL of HRP-conjugate 1X was added; it was agitated for 60 s, and

then incubated 40 min at 37°C. Washings were performed with the buffer for the corresponding times. The substrate was added to each well and incubated 20 min at 37°C. Added, were 50 μL of the stop solution with gentle agitation. The optical density was measured at 450 nm.

■ Statistical analysis

The data were analysed using SPSS software (Statistical Package for the Social Sciences, v.20, SPSS Inc., Chicago, IL). The Shapiro-Wilk test was used to determine the distribution of the study variables. The quantitative variables are expressed in mean ± standard or error deviation of the mean, and the Mann- Whitney U test was used to compare groups. The Wilcoxon test was used to compare the values before-after treatment. The qualitative variables are expressed in frequencies and percentages and were analyzed with Chi2 or Fisher exact test for the intra-group analysis. A value of p≤0.05 was considered statistically significant, with a confidence interval of 95%.

■ Ethical considerations

The study was conducted in agreement with guidelines as stipulated by the Declaration of Helsinki, 64th General Assembly in Fortaleza, Brazil in October 2013. The study was approved by the National Research and Ethics Committee of the Mexican Social Security Institute (R-2012-785-040) and by the Research Ethics and Bioethics Committee of the University Health Sciences Centre at the University of Guadalajara (C.I.2010). The Ministry of Health in the State of Jalisco authorized this research with registration: 62/UG-AL/2011. The study was recorded in ClinicalTrials.gov (NCT02062034). Patients were informed and informed consent forms were obtained and signed. Patient confidentiality was maintained at all times.

Results

■ Demographic and metabolic characteristics

The study groups were similar in demographic characteristics. The females predominated in Placebo group with 52.4% and 63.3% male in the Ubiquinone group (p=0.53). The average age was similar in Placebo group 58.2 ± 8.5 years and 58.8 ± 8.8 years old in the Ubiquinone group (p=0.932). There were no differences in weight between the groups; 76.8 ± 14.7 K in Placebo group vs. 73.7 ± 10.7 K in Ubiquinone

group (p=0.603). Placebo group were smoking-positive in 33% and 30% in the Ubiquinone group (p=0.71). The time of progression of the DM was >14 years in both groups (p=0.951). The baseline evaluations of the clinic parameters of systolic blood pressure (SBP), Diastolic Blood Pressure (DBP), glucose, HbA1c, Total cholesterol (TC), and Triglycerides (TG). The intraocular pressure (IOP) was similar to baseline values in both groups. The Ubiquinone group had significant decrease in DBP from baseline with 80.60 ± 10.30 mmHg to final 73.40 ± 12.00 mmHg (p=0.004). The HbA1c levels in the final results decreased in both groups; the baseline HbA1c in Placebo group was 9.20 ± 1.70% and final 8.10 ± 1.70% (p=0.008). In the Ubiquinone group, baseline HbA1c was 8.70 ± 1.70% and final 8.10 ± 1.70% (p=0.30). The baseline - final glucose in Placebo group was 125.2 ± 36.8 mg/dL - 135.3 ± 55.6 mg/dL. The glucose concentration in Ubiquinone group decreased at the end with 135.0 ± 48.2 mg/dL. The TC and TG concentrations in the final results decreased in both groups. The IOP did not show significant changes in the baseline - final determination in both groups; the Placebo group had 15.10 ± 2.10 mmHg at baseline, and 14.10 ± 4.00 mmHg (p=0.07) at the end. The Ubiquinone group showed 15.20 ± 1.80 mmHg as baseline, and 13.80 ± 4.90 mmHg as the final result (p=0.90) **(TABLE 1)**.

■ DNA damage and DNA enzymatic repair

The normal plasma level of 8-OHdG was 4.73 ± 0.96 ng/mL. The baseline levels in the Placebo group were significantly increased 6.9 ± 3.00 ng/mL (p=0.03) vs. normal control. The baseline Ubiquinone levels were also found to be significantly high compared to the normal level, 5.70 ± 1.50 ng/mL (p=0.045), interestingly the 8-OHdG decreased to normal levels at the end of the study, 4.60 ± 1.50 ng/mL (p=0.05).

The hOGG1 is the primary base-excision repair enzyme of the DNA that recognizes the 8-OHdG marker. Normal plasma levels were 0.39 ± 0.02 ng/mL. Baseline levels were significantly increased in both groups vs. normal levels: Placebo group, 0.43 ± 0.04 ng/mL (p=0.02) and Ubiquinone group, 0.43 ± 0.05 ng/mL. (p=0.003). Final results of this marker remained high in Placebo group 0.42 ± 0.03 ng/mL and a slight increase was observed in the Ubiquinone group, 0.50 ± 0.28 ng/mL (p=0.48) **(TABLE 2)**.

■ Endogenous antioxidants

The SOD and CoQ10 are endogenous antioxidants that favor re-establishment of the redox state in the organism. The normal plasma level of SOD was 10.20 ± 1.85 IU/mL. The baseline levels in the two study groups were significantly consumed vs. normal control: Placebo group, 6.02 ± 2.90 UI/mL (p=0.006), and the Ubiquinone group, 7.07 ± 4.10 UI/mL (p=0.01). At the end of the study: Plasma levels of SOD in Placebo group were even more consumed at 4.50 ± 3.30 IU/mL (p=0.02). The SOD levels improved in the Ubiquinone group with 8.26 ± 4.90 IU/mL (p=0.08) **(TABLE 2)**.

The normal plasma level of endogenous CoQ10 had 1295.20 ± 383.30 ng/mL. Baseline levels in both study groups were significantly diminished:

Table 1. Clinical and metabolic features. We observed significant decrease in diastolic arterial pressure at the final results in the group managed with CoQ10 (p=0.004). The HbA1c had a significant decrease at the end in Placebo group (p=0.008).

	Clinical and Metabolic Features					
	Placebo (n-20)			CoQ10 (n-20)		
	Basal	**Final**	*p*	**Basal**	**Final**	*p*
SBP, mm Hg	133.20 ± 17.70	129.50 ± 12.30	0.10	130.50 ± 16.40	132.40 ± 14.10	0.11
DBP, mm Hg	76.20 ± 8.80	75 ± 9.10	0.33	80.60 ± 10.30	73.40 ± 12.00	**0.004***
Biochemical features						
HbA1c, %	9.20 ± 1.70	8.10 ± 1.70	**0.008***	8.70 ± 1.70	8.10 ± 1.70	0.30
Glucose, mg/dL	125.2 ± 36.8	135.3 ± 55.6	0.40	149.0 ± 60.8	135.0 ± 48.2	0.37
Total cholesterol (TC), mg/dL	205.00 ± 32.40	185.90 ± 54.30	0.33	176.80 ± 34.80	168.00 ± 43.10	0.46
Triglycerides (TG), mg/dL	214.00 ± 102.60	180.40 ± 78.40	0.09	210.00 ± 133.70	197.40 ± 89.00	0.26
Ophthalmological features						
IOP, mm Hg	15.10 ± 2.10	14.10 ± 4.00	0.07	15.20 ± 1.80	13.80 ± 4.90	0.90

SBP: Systolic blood pressure; DBP: Diastolic Blood Pressure; IOP: Intraocular Pressure; HbA1c: Glycated Hemoglobin. Mean ± SEM p: Wilcoxon signed-rank test were used for comparison between baseline and final values.

Table 2. Markers of oxidative stress before and after the antioxidant treatment. Baseline 8-OHdG was significantly increased in the two study groups and decreased significantly at the final in the group treated with CoQ10.									
Oxidative Stress Markers before and after the Antioxidant Treatmesnt									
				Placebo (n-20)			**CoQ10 (n-20)**		
Marker	**Healthy Control**	**p UM**	**p UM**	**Baseline**	**Final**	**p WCX**	**Baseline**	**Final**	**p WCX**
Oxidative damage and repair DNA									
8-OHdG, ng/mL	4.73 ± 0.96	**0.03**	**0.045**	6.90 ± 3.00	6.80 ± 3.00	0.32	5.70 ± 1.50	4.60 ± 1.50	**0.05**
hOGG1, ng/mL	0.39 ± 0.02	**0.02**	**0.003**	0.43 ± 0.04	0.42 ± 0.03	0.43	0.43 ± 0.05	0.50 ± 0.28	0.48
Endogenous antioxidants									
SOD, UI/mL	10.20 ± 1.85	**0.006**	**0.01**	6.02 ± 2.90	4.50 ± 3.30	**0.02**	7.07 ± 4.10	8.26 ± 4.90	0.08
CoQ10, ng/mL	1295.00 ± 383.30	**<0.001**	**<0.001**	741.40 ± 152.00	808.90 ± 205.00	0.07	750.60 ± 82.00	1471.90 ± 164.00	**<0.001**

8-OHdG: 8-hydroxy-2'-deoxyguanosine; hOGG1: Human 8-oxoguanine-DNA-N-Glycosylase-1; SOD: Superoxide dismutase; CoQ10: Coenzyme Q10; UM: Mann Whitney U test was used for comparison between Healthy control vs. study group; WCX: Wilcoxon signed-rank test; were used for comparison between baseline-final values; K-W: Kruskall-Wallis test comparison.

Placebo group, 741.40 ± 152.00 ng/mL and Ubiquinone group, 750.60 ± 82.00 ng/mL (p<0.001 respectively). In the final results, CoQ10 levels in Placebo group increased slightly 808.90 ± 205.00 (p=0.07), and the Ubiquinone group the endogenous concentration increased to normal levels at the end, 1471.90 ± 164.00 ng/mL (p<0.001) **(TABLE 2).**

■ Adverse events

Adverse reactions reported were mild: nausea (2 patients) in Placebo group and insomnia (1 patient) in Ubiquinone group.

Discussion

The present study explored the antioxidant effect of Ubiquinone in relation to markers of the oxidative DNA damage and DNA repair in plasma in NPDR patients as well as the behavior of SOD activity and endogenous concentrations of CoQ10 as Antioxidants in the clinical outcomes in patients included in the study.

The properties of CoQ10 in the oxidative and non-oxidative damage to DNA were reported previously, where the protective effect of the CoQ10 on lymphocytes was demonstrated in studies in vitro [13]. We observed that after 6 months administration of 400 mg/day of CoQ10, the 8-OHdG the plasma levels decreased significantly (p=0.045) and levels of hOGG1 increased slightly. However, the mechanism by which the CoQ10 protects the genetic material from oxidative stress is not clear. One possible explanation could be the role that CoQ10 plays in the decrease in products of lipid peroxidation (Malondialdehyde, 4-hydroxynonenals) [16] and other free radicals as hydroperoxides, peroxyl and alcoxy radicals that have the capacity to oxidize and break the chains of DNA [4]. The hOGG1 enzyme is key in catalyzing the elimination of 8-OHdG [17]. The baseline - final concentration of hOGG1 was significantly higher in the Ubiquinone group (p=0.018), possibly in response to the increase in the 8-OHdG baseline levels. In the Ubiquinone group, we observed that baseline levels of SOD and CoQ10 were significantly diminished, which leads to the development of oxidative damage to macromolecules. This information relates to previous studies reported where the catalase, SOD, and glutathione peroxidase were decreased in DR vs. healthy subjects [18]. The benefit effect of CoQ10 on endogenous concentrations of antioxidants in coronary illness, and in the levels of SOD and catalase achieved after 12 weeks of ingestion as was previously reported [19]. In this study, baseline levels of CoQ10 were significantly diminished even in the final results, only the Ubiquinone group normalized their endogenous levels (p=0.0001). Measurement of CoQ10 in biological fluids is clinically important in the detection of DR if the deficiency of this co-enzyme exists [20]. Several clinical trials and case series have reported evidence that supports the complementary use of CoQ10 in prevention and treatment of diverse clinical disturbances. Our results demonstrate that after the administration of 400 mg/day of Ubiquinone for 6 months, we achieved an increase in endogenous CoQ10 to normal levels. Therefore, we would suggest that the values correspond as much to the

endogenous synthesis as well as for the exogenous administration, since it is widely known that after oral administration the CoQ10 rapidly converts it to reduced form, upon being oxidized in the membrane by the dependent NADPH enzymes in relation to demand and the oxidative state.

In order to affirm that the majority of the CoQ10 concentration is due to endogenous synthesis, we plan to evaluate levels of Ubiquinol in bodily fluids and demonstrate the Ubiquinol/ Ubiquinone ratio in DR and NPDR patients. The antioxidant activity of the CoQ10 is considered to have more synergy than other antioxidant vitamins, like vitamins C and A, as well as favoring the recycling of vitamins A, C and E [21], which makes CoQ10 an antioxidant with grand properties. For this reason, we consider that the consumption of CoQ10 demonstrate to be well-tolerated and safe, without serious adverse effects [22].

In conclusion; we suggest that the addition of Ubiquinone served as adjunctive to the treatment for metabolic control, since we observed reduction the oxidative DNA damage and increased the endogenous antioxidants. The limitations in the study are on the short treatment duration and the small sample size.

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