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Ubiquinone, Ezetimibe/Simvastatin and Rosuvastatin Effects on Mitochondrial Function in Diabetic Polyneuropathy

Luis M. Román-Pintos, Geannyne Villegas-Rivera,
Ernesto G. Cardona-Muñoz,
Adolfo D. Rodríguez-Carrizalez,
Fermín P. Pacheco-Moisés and
Alejandra G. Miranda-Díaz

Additional information is available at the end of the chapter

Abstract

Diabetic polyneuropathy (DPN) pathophysiologic findings include loss of multifocal and focal nerve fibers secondary to axonal degeneration and segmental demyelization due to oxidative stress and mitochondrial dysfunction induced by chronic hyperglycaemia.

Aim: To evaluate the effect of ubiquinone, ezetimibe/simvastatin and rosuvastatin on mitochondrial function in patients with diabetic polyneuropathy.

Methods: A randomized, double-blinded, placebo-controlled clinical trial was performed in patients with type 2 Diabetes Mellitus (T2DM) who had DPN, glycated haemoglobin (HbA1c) <12% (108 mmol/mol), previous exclusion of other type of neuropathy. Ninety-eight persons with T2DM were enrolled allocated 1:1:1:1 to either placebo, ubiquinone 400 mg, ezetimibe/simvastatin 10/20 mg or rosuvastatin 20 mg for 16 weeks. Primary outcomes were F0F1-ATP hydrolysis, erythrocyte and sub-mitochondrial platelet membrane fluidity. Results were expressed as mean ± SD or SEM and percentages.

Results: F0F1-ATP hydrolysis levels in healthy controls were 236.80±118.42 nmol/PO4; all patients with T2DM exhibited an increase, but only rosuvastatin demonstrated an improvement with baseline 463.37±47.07 nmol/PO4 vs. after 340.61±37.80 nmol/PO4 treatment (p<0.05). Plasma and sub-mitochondrial membrane fluidity did not experience any significant changes.

Conclusions: Rosuvastatin was found to improve mitochondrial function by reducing F0F1-ATP hydrolysis. No changes in sub-mitochondrial platelets particles or erythrocytes ghosts were found.

Keywords: ubiquinone, statins, diabetic polyneuropathy, oxidative stress, mitochondrial dysfunction

1. Introduction

Nerve dysfunction system in patients with diabetes is known as diabetic neuropathy and is considered as the most prevalent microvascular complication—up to 60%—in diabetes mellitus (DM) subjects [1]. Diabetic polyneuropathy (DPN) comprise ≈70% of all cases [2]. Diabetic sensorimotor polyneuropathy is the most common for these complications, occurring in patients with type 1 and 2 diabetes mellitus, as well as in those with prediabetes and glucose intolerance [3]. Its diagnosis is established by means of validated scores based on clinical features and abnormal nerve conduction studies (NCS) [4]. Pathophysiologic findings include loss of multifocal and focal nerve fibers secondary to axonal degeneration and segmental demyelization, basically due to oxidative stress and mitochondrial dysfunction induced by chronic hyperglycemia, which leads to neural apoptosis [5, 6]. Mitochondrial dysfunction leads to free radical excessive production through imbalance between hydrogen ions and electron transport carriers, which takes place in the inner mitochondrial membrane and could be evaluated indirectly by membrane fluidity and F0F1-ATPase hydrolysis [7, 8].

Ubiquinone (coenzyme Q10) is a potent antioxidant acting as an electron carrier in the mitochondrial electron transport chain, thus reducing free radical production with high concentrations present in high metabolic activity tissues such as heart, kidney, liver, and skeletal muscle [9]. Ezetimibe diminishes cholesterol ester content in chylomicrons by reducing liver cholesterol intake that in consequence increases LDL uptake and plasma depuration; when combined with simvastatin, cholesterol reduction is potentially increased [10]. Pleiotropic effects of statins include an increase in nuclear factor kappa B activity and amelioration of superoxide (O^{2-}) ions after 12-week treatment [11]. Another HMG-CoA inhibitor, rosuvastatin, has an antioxidant effect by acting as free radical carrier diminishing mitochondrial and cellular Lipid peroxidation (LPO) production [12].

We conducted this study to evaluate the effect of ubiquinone, ezetimibe/simvastatin, and rosuvastatin on mitochondrial function in patients with DPN.

2. Subjects, materials, and methods

2.1. Study design

A randomized, double blinded, placebo controlled phase II clinical trial was performed in the Clinic and Experimental Therapeutics Institute, University of Guadalajara, Mexico. Subjects

were assigned to four group treatments by blocks with a parallel sequence 1:1:1:1 by means of a randomized computer-based list, generated by a different researcher who was unaware of drugs given to patients. Patients were divided into the following groups: control group that received placebo and three experimental groups that were assigned to ubiquinone, ezetimibe/simvastatin, and rosuvastatin as a daily single dose for 16 weeks.

2.2. Study population

Participants were eligible based on Dyck et al. characteristics for DPN [4]. Inclusion criteria were subjects ≥18 years old, T2DM (T2DM) according to ADA (American Diabetes Association) criteria, HbA1c <12%, and informed consent signed. Patients were excluded if they had renal or hepatic failure, pregnant or nursing women, and other neuropathies rather than diabetic (alcohol-induced, radiculopathy, autoimmune, cancer-related). They were eliminated once admitted if lack of adherence to treatment evaluated when <80% of drug intake, and/or severe adverse drug reaction. Patients were selected by invitation in forums, outpatients recruited from primary care clinics, and database collected previously by our research Institute, Guadalajara residents from February 2010 to 2012. Patients were instructed to take their drugs only by night at the same time every day as follows: placebo 100 mg, ubiquinone 400 mg, ezetimibe/simvastatin 10/20 mg, and rosuvastatin 20 mg. All drugs were similar in physical characteristics and presented in dark vials, carefully filled by another group researcher who placed a respective tag with the patient code. Moreover, patients were provided with a diary where they could write down the date and time of drug administration, and drug adverse reactions felt. Such information was collected and registered after every 4 weeks. Primary outcomes were mitochondrial function parameters: F0F1-ATPase hydrolysis, erythrocyte ghosts, and submitochondrial platelet membrane fluidity. We also measured other metabolic and safety measures: fasting glucose, HbA1c, total cholesterol, high and low density lipoproteins (HDL, LDL), and triglycerides. Safety profile was assessed with drug adverse reactions, renal (urea, creatinine), and hepatic [alanine and aspartate aminotransferase (ALT, AST), gamma glutamil-transferase (GGT), and bilirubin and phosphokinase (CPK)] laboratory variables.

2.3. Healthy subjects

We included an additional group consisting of nine subjects randomly selected from a preventive medicine first contact clinic to establish a cutoff point for the below-mentioned parameters. Healthy age and sex-matched volunteers without DM were included in the control group.

2.4. Mitochondrial function markers

Hydrolytic activity of mitochondrial F0F1-ATPase was obtained by spectrophotometry, from release of inorganic phosphate in serum samples of platelets isolated according to the method described by Baracca et al. [13]. Thirty microliters of the sample and 20 μL of ATP (100 mM) were added to 1 mL of ATPase buffer [125 mM KCl, 40 mM of Mops (pH 8), 3 mM MgCl2], and then agitated, followed by incubation at 40°C for 10 minutes. ATPase activity was stopped

with 200 μL of 30% trichloroacetic acid, and samples were centrifuged for 10 minutes at 3500 rpm. Then, 1 mL of 3.3% ammonium molybdate and 100 μL of 10% ferrous sulfate were added to 800 μL of the supernatant, and samples were incubated for 20 minutes at room temperature to finally read absorbance at 660 nm. Results are expressed in nmol/PO4.

Membrane fluidity of erythrocyte ghosts and submitochondrial membrane in platelets was performed once obtained by centrifuging whole blood from patients at 4°C for 15 minutes at 3500 rpm. Supernatant was removed from the residue and 200 μL of cold buffer was added (NaCl 140 mM, KCl 4.7 mM, MgCl 1.2 mM, KH_2PO_4 1.2 mM, dextrose 11 mM and HEPES 15 mM), and homogenized. Platelets (70 μL) were isolated and stored at −80°C. Evaluation of membrane fluidity was performed by incorporation of 1,3-dipyrenylpropane (DPyP) to biological membranes [14]. Two milliliters of Tris-HCL buffer with a pH of 7.8 (10 mM) was added to 35 μL of the mitochondrial membranes and mixed at 24°C. Using fluorescent spectrophotometer (Perkin Elmer L550B), the fluorescent intensity of the monomer (Im) and excimer (Ie) were measured at 395 and 494 nm, respectively. Immediately, 5 μL (0.1 μg) of DPyP was added and incubated at room temperature in darkness for 3 hours, in order to allow the incorporation of the DPyP to membranes. The second measurement was performed at the same wavelengths and the Ie/Im ratio of the fluorescence intensity was calculated.

2.5. Ethical considerations

Study was approved by the Research and Ethics Committee of the Health Science University Center, University of Guadalajara, Mexico. Identification codes were assigned to each participant to guarantee patient confidentiality, and an informed consent form was signed before entering the protocol, according to national and international laws (National Institutes of Health) with clinical trial identifier NCT02129231 and also as stipulated by the Helsinki Statements in 2000.

2.6. Statistical analysis

Sample size determination was described elsewhere [15] taking into account a 95% confidence interval, 80% potency, and two-tailed $p < 0.05$, which resulted in 21 for each group. Quantitative variables were expressed as mean ± standard deviation. Wilcoxon tests were realized before and after measurements, and Kruskal-Wallis with Mann-Whitney′s U test as post hoc analysis for between-group comparisons. Qualitative variables were expressed as frequencies and percentages. The McNemar test was used to evaluate differences in dichotomy variables before and after treatment, between-group comparisons were determined by Fisher′s exact test and χ^2 as needed. The significance level was established with p-value <0.05.

3. Results

In the scrutiny phase, 155 patients were assessed, of which 63 were not eligible and 98 were included as follows: placebo 24, ubiquinone 24, ezetimibe/simvastatin 25, and rosuvastatin 25 (**Figure 1**).
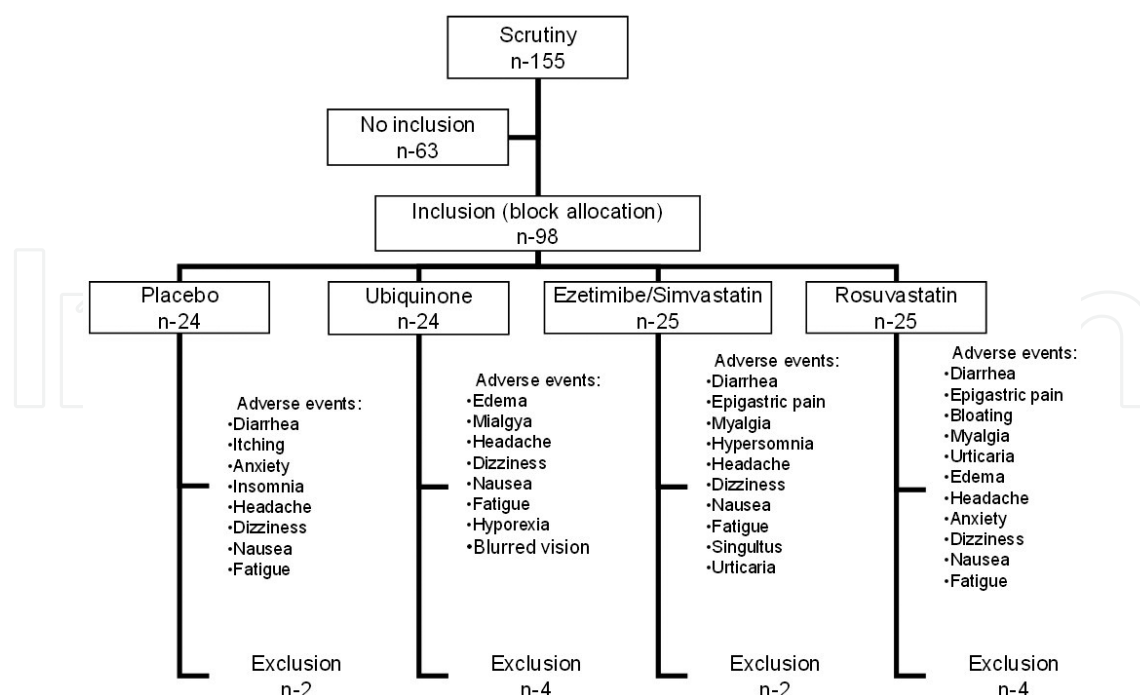
Figure 1. Flow diagram for patients follow-up.

Demographic features were homogeneous at baseline without significant differences between groups. Notably, there were a greater number of women above 50 years old and more than 10 years with T2DM. Overweight and obesity was found in all treatment groups (**Table 1**).

3.1. Mitochondrial function markers

F0-F1-ATPase in HS was 236.80 ± 118.42 nmol/PO4, those with placebo had 416.23 ± 38.39 nmol/PO4 ($p = 0.094$ vs. HS), for ubiquinone 535.86 ± 65.14 nmol/PO4 ($p < 0.05$ vs. HS), ezetimibe/simvastatin 447.09 ± 56.91 nmol/PO4 ($p = 0.065$ vs. HS), and rosuvastatin with 463.37 ± 47.07 nmol/PO4 ($p < 0.05$ vs. HS). There was no significant difference between treatment groups ($p = 0.583$, Kruskal-Wallis). At the end of treatment, placebo group had 443.41 ± 42.86 nmol/PO4 ($p = 0.783$, baseline vs. final), ubiquinone 391.30 ± 42.08 nmol/PO4 ($p = 0.823$, baseline vs. final), ezetimibe/simvastatin 328.50 ± 36.38 nmol/PO4 ($p = 0.426$, baseline vs. final), and rosuvastatin group had a significant reduction with 340.61 ± 37.80 nmol/PO4 ($p < 0.05$, baseline vs. final; **Figure 2A**).

Erythrocyte ghost membrane fluidity in HS was 0.80 ± 0.19 Ie/Im, while baseline levels on placebo group were 0.97 ± 0.10 Ie/Im ($p = 0.293$ vs. HS), ubiquinone 1.05 ± 0.18 Ie/Im ($p = 0.571$ vs. HS), ezetimibe/simvastatin had 1.23 ± 0.30 Ie/Im ($p = 0.936$ vs. HS), and rosuvastatin 0.98 ± 0.19 Ie/Im ($p = 0.936$ vs. HS); moreover, no significant differences were observed between treatment groups ($p = 0.632$, Kruskal-Wallis). After treatment period, there was no evident change in either group with placebo 1.13 ± 0.31 Ie/Im ($p = 0.836$, baseline vs. final), ubiquinone 1.21 ± 0.25 Ie/Im ($p = 0.245$, baseline vs. final), ezetimibe/simvastatin 1.14 ± 0.19 Ie/Im ($p = 0.983$ baseline vs. final), and rosuvastatin 0.99 ± 0.17 Ie/Im ($p = 0.778$, baseline vs. final), without

difference between groups (p = 0.837, Kruskal-Wallis). There was no change in membrane fluidity after 16-week treatment, probably due to short-period intervention or this particular membrane property is not involved y pathogenic alterations noted in DM patients (**Figure 2B**).

	Placebo (n=24)	Ubiquinone (n=24)	Ezetimibe/ Simvastatin (n=25)	Rosuvastatin (n=25)
Gender (M/F), n (%)	7/17 (29/71)	9/15 (38/62)	10/15 (40/60)	12/13 (48/52)
Age (years)	54.7±9.6	58.8±9.2	55.0±12.0	54.0±10.5
Weight (kilograms)	73.7±11.4	73.8±8.6	75.4±13.9	76.9±18.7
Height (meters)	1.59±0.09	1.59±0.11	1.60±0.10	1.62±0.13
BMI (kg/m²)	29.3±4.3	29.3±7.3	29.4±4.1	29.0±4.7
Type 2 DM duration (years)	10.5±8.3	9.7±6.2	10.2±6.6	12.1±8.3
SBP (mmHg)	142±25	134±20	144±25	135±17
DBP (mmHg)	84±11	78±8	81±10	81±7
Smoking (Y/N), n (%)	9/15 (38/62)	10/14 (42/58)	8/17 (32/68)	12/13 (48/52)
Type 2 DM treatment, n (%)				
Insulin	1 (4.2)	1 (4.2)	2 (8.0)	
Metformin	5 (20.8)	5 (20.8)	5 (20.0)	4 (16.0)
Glyburide		1 (4.2)	1 (4.0)	3 (12.0)
Metformin/Glyburide	15 (62.5)	12 (50.0)	13 (52.0)	11 (44.0)
Metformin/Insulin	1 (4.2)	3 (12.5)	2 (8.0)	2 (8.0)
Metformin/Glyb/Insulin		1 (4.2)		3 (12.0)
Others	2 (8.3)	1 (4.2)	2(8.0)	2 (8.0)

Values in mean ± SD, unless otherwise specified.
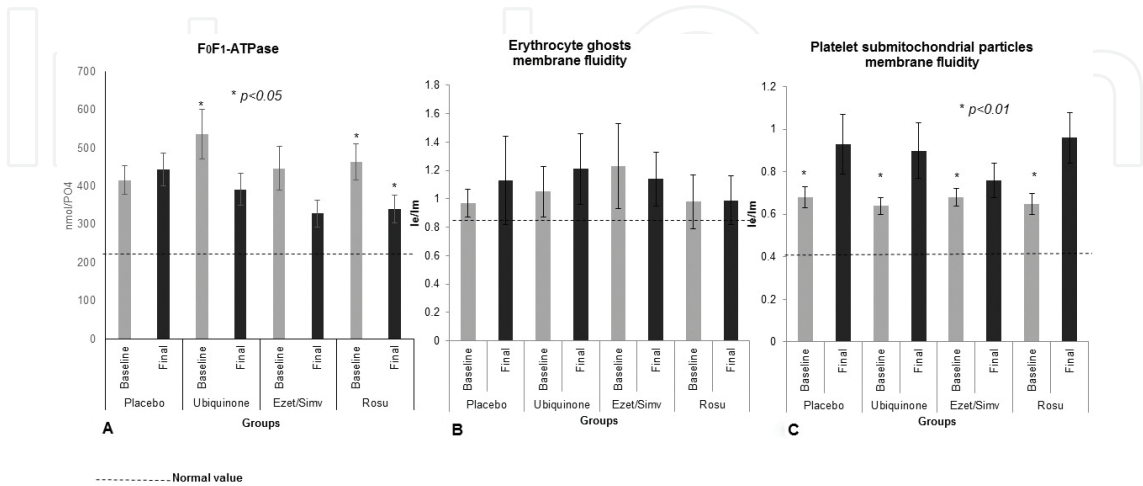
Table 1. Demographic and clinical features.

Figure 2. Mitochondrial dysfunction parameters in patients with DPN.

Submitochondrial platelet membrane fluidity in HS had 0.38 ± 0.03 Ie/Im, with increase in treatment groups; placebo control group had 0.68 ± 0.05 Ie/Im ($p < 0.01$ vs. HS), ubiquinone 0.64 ± 0.04 Ie/Im ($p < 0.01$ vs. HS), ezetimibe/simvastatin 0.68 ± 0.04 Ie/Im ($p < 0.01$ vs. HS), and rosuvastatin 0.65 ± 0.05 Ie/Im ($p < 0.01$ vs. HS); however, no statistical difference between treatment groups was found ($p = 0.675$, Kruskal-Wallis). After 16-week treatment there was no significant change in any group, those with placebo had 0.93 ± 0.14 Ie/Im ($p = 0.426$, baseline vs. final), ubiquinone 0.90 ± 0.13 Ie/Im ($p = 0.057$, baseline vs. final), ezetimibe/simvastatin 0.76 ± 0.08 Ie/Im ($p = 0.670$, baseline vs. final), and rosuvastatin 0.96 ± 0.12 Ie/Im ($p = 0.092$, baseline vs. final), without difference between groups ($p = 0.780$, Kruskal-Wallis). There is a significant difference in submitochondrial membrane fluidity when compared with healthy controls, probably because of underlying pathophysiologic changes in patients with diabetes, which cannot be resolved with short-time treatment (**Figure 2C**).

3.2. Metabolic and safety profile parameters

Serum transaminases were increased on experimental groups at baseline compared with placebo, without clinical relevance. A significant reduction in fasting glucose was noted for placebo and ubiquinone groups, probably due to lifestyle changes; however, no difference on HbA1c was observed. A reduction in the total bilirubin on ubiquinone and rosuvastatin treatments was also noted (–0.16 ± 0.27 and –0.17 ± 0.31 mg/dL, respectively), without significant difference compared with placebo. As expected, a significant reduction in TC, LDL, and TG was detected on statins (CT-82.8 ± 49.8 mg/dL and LDL-57.1 ± 48.4 mg/dL for ezetimibe/simvastatin ($p < 0.001$), and CT-73.3 ± 49.5 mg/dL, LDL-64.9 ± 44.0 mg/dL, and TG-41.1 ± 61.2 mg/dL) ($p < 0.001$, $p < 0.001$ and $p < 0.01$, respectively vs. placebo) for rosuvastatin (**Table 2**). Gastrointestinal, neurologic, dermatologic, and muscular adverse events related to drugs were reported, but only two patients were eliminated from the study for myopathy related to statins (one with ezetimibe/simvastatin and the other with rosuvastatin).

	Placebo (n=24)			Ubiquinone (n=24)			Ezetimibe/ Simvastatin (n=25)			Rosuvastatin (n=25)			P (Kruskal-Wallis)
	Base line	Final	p	Base line	Final	p	Base line	Final	p	Base line	Final	p	
Glucose (mg/dL)	186.4± 14.48	150.2± 11.2	**0.004**	159.5± 11.05	138.1± 11	**0.020**	146.64± 9.92	152.6± 14	0.620	192.04± 12.73	179.2± 15.2	0.520	0.220
Urea (mg/dL)	29.5± 2.1	28.9± 2.2	0.520	29.23± 1.93	30.5± 2.5	0.170	30.03± 1.51	32.5± 1.9	0.280	31.22± 2.27	28.6± 2.2	0.300	0.440
Creatinine (mg/dL)	0.79± 0.04	0.84± 0.05	0.180	0.81± 0.05	0.80± 0.05	0.840	0.82± 0.04	0.85± 0.05	0.570	0.84± 0.04	0.80± 0.04	0.320	0.750
AST (U/L)	21.9± 1.89	21.8± 1.4	0.820	27.22± 2.5	**28**± **2.4***	0.400	24.63± 0.99	**27.1**± **2.5***	0.270	27.8± 1.91	**26.7**± **1.2^**	0.700	**0.020**

	Placebo (n=24)			Ubiquinone (n=24)			Ezetimibe/Simvastatin (n=25)			Rosuvastatin (n=25)			P (Kruskal-Wallis)
	Base line	Final	p	Base line	Final	p	Base line	Final	p	Base line	Final	p	
ALT (U/L)	20.2± 1.43	20.5± 1.9	0.990	29.5± 4.88	**30.3± 3.3***	0.730	28.57± 3.26	**28.4± 3.1***	0.570	31.22± 2.47	**30.3± 2.4^**	0.870	**0.010**
GGT (U/L)	34.73± 6.49	39.4± 8.6	0.270	55.22± 12.98	59.6± 17.9	0.600	39.83± 9.04	33.7± 3.7	0.780	44.33± 6.93	43.9± 7.7	0.260	0.800
Total Bilirubin (mg/dL)	0.70± 0.06	0.56± 0.05	0.052	0.77± 0.06	0.63± 0.08	**0.030**	0.61± 0.04	0.64± 0.06	0.800	0.83± 0.05	0.65± 0.07	**0.020**	0.790
Direct bilirubin (mg/dL)	0.11± 0.01	0.11± 0.01	0.780	0.12± 0.02	0.15± 0.03	0.290	0.10± 0.01	0.13± 0.01	0.080	0.15± 0.02	0.13± 0.02	0.420	0.830
Total cholesterol (mg/dL)	211.43 ±11.73	202.3 ±8	0.610	230.17 ±9.89	227.1± 8.3	0.330	210.56 ±9.94	**129.3± 9.7¥**	0.001	217.2± 8.04	**142.7± 8.8¥**	0.001	**0.001**
Low density cholesterol (mg/dL)	126.68 ±8.76	109.6± 7.8	**0.010**	134.19 ±8.08	132.5± 8.8	0.790	117.45 ±7.16	**61.7± 6.1¥**	0.001	133.56 ±7.96	**75± 8.1¥**	0.001	**0.001**
HDL cholesterol mg/dL)	36.95 ±2.61	39.7± 2.5	0.470	43.95± 3.69	43.4± 3.9	0.300	36.23± 2.06	32.7± 2.1	0.090	36.79± 1.91	36.5± 2.3	0.360	0.060
Triglycerides (mg/dL)	240.13 ±27.41	242.4	0.590	260.25 ±36.59	263.1± 37.9	0.550	234.63 ±49.96	**161.9± 21.2***	0.055	220.6± 24.49	**168.4± 24.4***	0.003	**0.005**
CPK (U/L)	82.21± 13.78	82.8± 10.3	0.930	101.74± 18.42	00± 13.1	0.990	95.92± 14.4	86.4± 8.3	0.460	114.93 ±18.44	157.3± 35.1	0.190	0.100
A1c (%)	8.83± 0.36	9.2± 0.5	0.260	8.49± 0.36	7.9± 0.3	0.090	7.84± 0.32	8.1± 0.4	0.250	8.97± 0.40	**9.4± 0.4∞**	0.090	**0.030**

Median ± Standard error *$p<0.05$ ^$p<0.01$ ¥$p<0.001$ vs. Placebo ∞$p<0.01$ vs. Ubiquinone (Mann-Whitney´s U test). A1c, glycated hemoglobin; ALT, alanine aminotransferase; AST, aspartate aminotransferase; CK, creatinine kinase; GGT, gamma glutamyl transferase; HDL, high-density cholesterol; LDL, low density cholesterol.

Table 2. Metabolic characteristics.

4. Discussion

Oxidative stress generates many of the pathophysiologic changes found in T2DM [16]; furthermore, free radical excessive production is involved in axonal degeneration and segmental demyelinization found in T2DM patients with DPN [17, 18]. Mitochondrial biogenesis is a complex process that involves more than 100 proteins coded by the nucleus and requires coordination with synthesis of 13 proteins coded by mitochondrial DNA. The integrity of this process is essential for normal oxidative phosphorylation, primary source of ATP production, O_2-, H_2O_2, and other ROS, which plays a fundamental role in the initiation and progression of diabetes. These products can overregulate the expression of proinflammatory cytokines and unchain death signals [19]. Mitochondria are very efficient by using glucose substrates to produce energy in the form of ATP. In this process, protons are pumped from the mitochondrial matrix to cross the inner mitochondrial membrane through the respiratory complexes forming the oxidative phosphorylation chain. Five complexes are implicated in ATP synthesis, culminating in complex V or ATP-synthase, and an inverse process occurs at the same time, named as ATP hydrolysis. An increase in F0F1-ATP hydrolysis has been found in rats with induced DM, probably induced by mitochondrial dysfunction [20], just as seen in treatment groups before intervention. Rosuvastatin demonstrated a reduction in F0F1-ATP hydrolysis after 16-week period, probably by reducing free radical production through improvement of NAD(P)H oxidase [21], constituting the first study to report this finding. We suppose an improvement in mitochondrial function by reduction in its catabolism, and probably reducing cell apoptosis.

The most important functions of erythrocyte membrane include (a) enzymatic activity, (b) ion transport of ions and anionic substances, (c) osmotic stability, (d) oxygen diffusion, and (e) membrane receptor activity, where a change in elasticity results in the deterioration of blood flow and worsens tissue perfusion [22]. Membrane fluidity of erythrocyte ghosts was slightly increased in patients with diabetes compared with HS. After intervention, all patients maintain almost the same values, probably due to T2DM chronicity, short-time treatment period, and/or not enough doses to improve this membrane property. In 2002, Koter et al. demonstrated a reduction in membrane fluidity with atorvastatin after 12 weeks of treatment in 31 hypercholesterolemic subjects [23]; however, T2DM consists in more complex physiopathologic components than lipid alterations as a single insult. More profound research must be conducted to establish the exact mechanisms that can be restored in order to improve erythrocyte membrane fluidity.

Submitochondrial particles are primarily composed of the internal mitochondrial membrane where oxidative phosphorylation and other enzymes are involved as metabolite carriers. Patients with T2DM had upper levels of platelet submitochondrial membrane fluidity when compared with HS, along with an increase after intervention. Until now, this was the first time to measure this particular platelet property. So, it is difficult to make a statement concerning its role on the pathogenesis of DPN. Previous studies on Alzheimer disease has shown low levels of platelet submitochondrial membrane fluidity due to increased levels of LPO [13]. So,

we could speculate that an improvement in the mitochondrial function was achieved after treatment in all groups; however, no significant differences were found between them.

Metabolic outcomes were as expected, whereas no changes were observed on placebo and ubiquinone groups using statin-reduced TC, LDL, and TG. A recently published article (IM-PROVE-IT) showed that ezetimibe combined with simvastatin enhances the lipid-lowering effect of this statin monotherapy, with the same security [24]. This study highlights the importance of LDL reduction in the improvement of cardiovascular events. Rosuvastatin showed a more intense reduction in LDL and TG, with an additional effect on F0F1-ATP hydrolysis, which raises the concern about the implication of oxidized LDL in the pathophysiology of T2DM microvascular complications.

A slight decrease in HbA1c was shown before and after intervention with ubiquinone, not enough to have a statistical inference but probably a larger number of patients and/or longer period of treatment would be needed to have a significant result. On the other hand, both placebo and ubiquinone had significant changes in fasting plasma glucose after treatment, probably due to tight control on lifestyle modifications. Finally, a slight increase in transaminases was observed with the three experimental groups compared with placebo, but levels were at normal range and there was no difference before and after 16-week intervention. So, it was considered as an incidental finding without clinically correlation.

In conclusion, patients with T2DM exhibit an increase in F0F1-ATP hydrolysis and membrane fluidity in erythrocytes and platelets, probably due to hypercatabolism secondary to hyperglycemia. A decreased mitochondrial membrane potential, dysfunction in intramitochondrial calcium regulation, and depletion of ATP production have been described previously; however, this is the first time, to our knowledge, that an increase in ATP hydrolysis is reported. These mechanisms, collectively, may lead to axon degeneration [19].

Alpha-lipoic acid (ALA) is the only effective treatment for neuropathological changes in DPN; for responders to initial 4-week high-dose (600 mg tid) administration of ALA, a subsequent treatment with ALA (600 mg qd) over 16 weeks effectively diminished neuropathic symptoms, whereas ALA withdrawal was associated with a higher use of rescue analgesic drugs in type 2 diabetic patients with symptomatic DPN [25]. However, ubiquinone and statins have proved a reduction in the oxidative stress status and neuropathic pain relief in previous publications [15, 26, 27].

We now demonstrate that all treatment groups had a diminishing effect on F0F1-ATP hydrolysis, but only rosuvastatin was found to improve mitochondrial function by significantly reducing F0F1-ATP hydrolysis. No changes in submitochondrial platelet particles or erythrocyte ghosts were found with this 16-week period treatment with ubiquinone, ezetimibe/simvastatin, or rosuvastatin; more research needs to be conducted to avoid bias, such as heterogeneity of antidiabetic drugs, lifestyle changes during the study, and reduced treatment period.

Author details

Luis M. Román-Pintos[1], Geannyne Villegas-Rivera[2], Ernesto G. Cardona-Muñoz[3],
Adolfo D. Rodríguez-Carrizalez[3], Fermín P. Pacheco-Moisés[4] and
Alejandra G. Miranda-Díaz[3*]

*Address all correspondence to: kindalex@outlook.com

1 Tonalá University Centre, University of Guadalajara, Guadalajara, Jalisco, México

2 Western Clinical Research Institute, Guadalajara, Jalisco, México

3 Clinical and Experimental Therapeutics Institute, Department of Physiology, Health Sciences University Centre, University of Guadalajara, Guadalajara, Jalisco, México

4 Department of Pharmacobiology, Engineering and Exact Sciences University Centre, University of Guadalajara, Guadalajara, Jalisco, México

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