Effect of short-term carvedilol therapy on salivary and plasma oxidative stress parameters and plasma glucose level in type II diabetes

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Background: Type II diabetes is associated with oxidative stress while carvedilol has been shown to have antioxidant properties, which are thought to account for the protective effects. **Objective:** The objective of this study was to compare the short-term effects of carvedilol versus placebo on oxidative stress status in type II diabetic patients. **Methods:** A total of 40 patients were randomly allocated to receive carvedilol (6.25 mg three-times daily; n = 20) or placebo (n = 20) for 2 weeks. Fasting blood sugar, hemoglobin A1c, total antioxidant capacity (ferric-reducing ability of plasma test) and lipid peroxidation (thiobarbituric acid reactive substances assay) in plasma and saliva were measured before and after the intervention. **Results:** No significant difference in oxidative stress status, fasting blood sugar and hemoglobin A1c was observed among the placebo- and carvedilol-treated groups. **Conclusion:** We conclude that unlike long-term therapy, short-term therapy with carvedilol is incapable of exerting its antioxidant properties and reducing blood sugar levels.

Keywords: blood, carvedilol, fasting blood sugar, hemoglobin A1c, lipid peroxidation, saliva, total antioxidant power, type II diabetes

Oxidative stress is a situation in which the amount of reactive oxygen species (ROS) exceeds the levels of neutralizing substances referred to as antioxidants. Type II diabetes is associated with oxidative stress and there is compelling biochemical evidence that suggests that ROS may play a role in the pathogenesis of type II diabetes [1–4]. These observations have provided sufficient impetus for the use of antioxidant supplements as adjunct therapy for the control of blood sugar levels in diabetic patients. However, there is currently no optimum regimen of antioxidant supplementation for diabetic patients [5].

Diabetes is frequently associated with heart failure and is an independent risk factor for increased mortality and morbidity. β-blockers are traditionally regarded as relatively contraindicated in patients with diabetes mellitus. Carvedilol administration has been associated with improvement in left ventricular function, clinical symptoms and hemodynamic parameters compared with baseline, for both diabetic and nondiabetic patients [6]. Carvedilol is an adrenergic antagonist with nonselective β- and α1-receptor-blocking properties. Recently it has received much attention due to its antiarrhythmic, antiapoptotic and inotropic effects as well as its capability to reduce the rate of mortality in heart failure patients [7]. Carvedilol tolerability in diabetic patients has been approved in several trials [8,9] and surprisingly, in contrast with adverse effects of other β-blockers in diabetic patients on glucose and lipid metabolism, carvedilol increases peripheral insulin sensitivity [10–12]. In addition, carvedilol acts as a potent antioxidant due to the unique carbazol moiety contained in its structure [13,14]. It may directly inhibit oxidative stress by scavenging ROSs or reducing their generation through sequestration of the ferric ions needed for the nonenzymatic production of hydroxyl radicals [14,15]. Several *in vitro* and animal studies have shown promising evidence about the antioxidant effect of carvedilol in oxidatively stressed models [19–24]; however, clinical trials in this field are scarce and controversial [25–33] and there is no clinical trial considering diabetes and the antioxidant effect of short-term carvedilol therapy. Therefore, in a short-term, double-blind, randomized, controlled trial, we investigated the antioxidant properties of carvedilol versus placebo, by assessing salivary and plasma oxidative stress status before and after treatment.

Methods

Participants & study design

This randomized, double-blind, placebo-controlled, clinical trial was performed on Iranian patients with type II diabetes in the out-patient clinic of the Endocrine and Metabolism Research Center of Tehran University of Medical Sciences (TUMS). Subjects were previously diagnosed patients with diabeties according to World Health Organization (WHO) criteria which were controlled by diet and oral hypoglycemic therapy.

Exclusion criteria included proliferative retinopathy, significant renal impairment (serum creatinine > 270 µmol/l), coronary artery disease,

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chronic liver disease, diabetic foot ulceration and gangrene, pulmonary infection, allergy to β-blockers, decompensated heart failure, heart block type II and III, severe bradycardia, smoking, drinking, pregnancy, age less than 20 or over 50 years, periodontal disease and supplementation with multivitamins or traditional herbs in the previous 3 months.

The Ethics committee of TUMS approved the study protocol. All subjects gave written, informed consent before participating in the study. A total of 40 subjects were randomly assigned into two groups. One group (n = 20) received 6.25 mg carvedilol three times a day for 14 days and the other (n = 20) received placebo. Follow-up examinations took place once every week at the outpatient clinic of the Endocrine and Metabolism Research Center of TUMS.

As age, exercise, smoking, polyunsaturated fat intake and dietary antioxidant vitamin intakes can affect oxidative status, subjects were given specific guidelines to follow throughout the study. The subjects were instructe not to take any multivitamin supplements or traditional herbs, and to consume no more than two servings of vegetable oil, three of fruit and five of vegetables daily. In addition, it was recommended that the subjects engage in low-impact exercise for 30 min/day.

Blood sample collection

Fasting blood was collected in tubes containing ethylenediaminetetraacetic acid (EDTA)–calcium complex before and 2 weeks after the administration of carvedilol or placebo. After centrifugation of the blood at 3000 g for 30 min at 4°C, the plasma supernatant fluid was separated and stored at -80°C until analyzed further.

Saliva sample collection

Unstimulated whole saliva (~3 ml) was collected, allowed to drain into a plastic container and centrifuged at 10,000 g, 4°C for 5 min to remove bacterial and cellular debris. Saliva samples were stored at -80°C until analysis.

Total antioxidant power assay

Antioxidant power of saliva and plasma was found by measuring their ability to reduce ferric ion on the basis of the ferric-reducing ability of plasma (FRAP) test [16]. The produced ferrous with the reagent tripyridyltriazine (TPTZ) produces a blue color with absorbance at 593 nm.

Lipid peroxidation assay

In this test, the lipid peroxide products, specifically malondialdehyde (MDA) which reacts with thiobarbituric acid (TBA), and is determined spectrophotometrically. Lipid peroxidation samples are assessed in terms of the TBA-reactive substances (RS) produced [17]. The 1,1,3,3-tetraethoxypropan standard solution was used to determine the concentrations of TBARS in samples.

Measurement of blood hemoglobin

Hemoglobin (Hb)A1c was measured by high performance liquid chromatography (HPLC) as described previously [18].

Statistical analysis

The statistical analysis was carried out using SPSS version 11.5. Continuous data were expressed as means ± standard error. General characteristics such as age, sex, duration of diabetes, body mass index and blood-glucose concentrations were compared between groups with the use of unpaired two-sample Student's t-test for continuous data and Chi-square test for categorical data (if applicable). A paired t-test was applied to compare before and after intervention levels of favorable parameters in each group. A p-value of less than 0.05 was considered to be significant.

Results

Table 1 shows baseline characteristics of the subjects in both groups. There was no significant difference between these characteristics.

Regarding changes in oxidative stress parameters before and after our intervention, Table 2 shows the amounts of these factors in the carvedilol and

Table 1. Baseline characteristics of subjects.

Characteristics	Treated with carvedilol	Treated with placebo
Age (years)	49.72 ± 8.45	50.63 ± 1.49
Gender (%)	31 (M), 68 (F)	31 (M), 68 (F)
Diabetes duration (years)	6.58 ± 1.54	6.35 ± 1.24
FBS (mg/dl)	209.42 ± 29.84	168.05 ± 17.37
HbA1c (%)	8.71 ± 0.74	7.39 ± 0.47

F: Female; FBS: Fasting blood sugar; Hb: Hemaglobin; M: Male.

Table 2. Levels of TBARS, total antioxidant capacity, FBS and HbA1c before and after the administration of carvedilol or placebo.

Characteristics	Carvedilol		Placebo	
	Before	*After*	*Before*	*After*
Blood TBARS (mmol/l)	1.07 ± 0.11	0.82 ± 0.07*	1.18 ± 0.08	0.80 ± 0.06*
Saliva TBARS (mmol/l)	2.22 ± 0.10	1.96 ± 0.12*	2.29 ± 0.13	1.94 ± 0.07*
Blood FRAP (mmol/l)	197.15 ± 18.74	154.46 ± 18.4*	205.49 ± 13.98	174.41 ± 16.48*
Saliva FRAP (mmol/l)	146.25 ± 15.96	100.90 ± 14.52*	130.24 ± 19.28	72.99 ± 10.49*
FBS	208.77 ± 20.14	185.00 ± 17.68	149.61 ± 17.40	142.15 ± 12.16
HbA1c	8.41 ± 0.74	8.55 ± 0.51	7.01 ± 0.50	7.38 ± 0.43

FBS: Fasting blood sugar; FRAP: Ferric-reducing ability of plasma; Hb: Hemoglobin;
TBARS: Thiobarbituric acid reactive substances.
**Significant difference between before and after intervention; $p < 0.05$.*
There is no significant difference in measured variables among carvedilol- and placebo-treated subjects.

placebo groups. It also demonstrates the HbA1c and fasting blood sugar (FBS) levels of the patients at the beginning and end of the study. Carvedilol was effective in reducing total antioxidant power significantly in saliva or blood; however, this effort was not observed for lipid peroxidation. Table 2 also shows that the placebo had an effect on total antioxidant power and lipid peroxidation both in saliva and blood. No significant difference was observed between the carvedilol and placebo group, either in total antioxidant capacity or lipid peroxidation in saliva or blood. HbA1c and FBS levels did not change throughout the study, either in the case or placebo group.

Discussion

In vitro carvedilol is believed to halt the destructive effects of ROS [19–21]. Almost all of the animal studies investigated the short-term effect of carvedilol against oxidative stress, and achieved a decrement of ROS and an increment of antioxidant capacity [22–24]. Human studies in this field can be categorized into short-term (1–8 weeks) and long-term (4–9 months) studies. Among the long-term studies, five clinical trials have considered antioxidant effects of carvedilol in essential hypertension [25], type II diabetes [26], cyclosporin-induced hypertension [27], dilated cardiomyopathy [28] and heart failure patients [29]. Although these studies used different methods for measuring oxidative stress, improvement in oxidative stress has been evident in all. In short-term studies, a 6-day randomized, double-blind, clinical trial on healthy subjects showed no significant change in markers of oxidative stress in their subjects [30]. The same results were achieved in another trial on heart failure patients who had undergone carvedilol therapy for 2 months [31]. Similar to short-term studies, compared with placebo, we did not observe any improvement in oxidative stress status in our diabetic patients treated with carvedilol for 14 days.

Conclusion

Considering our results and the trials discussed above, we conclude that carvedilol exerts its antioxidant effects in long-term treatment – approximately 3 months. However, larger longitudinal studies should be performed to confirm this assumption. Amazingly, the same scenario occurs concerning FBS levels in patients with diabeties treated with carvedilol. Two short-term trials with diabetic patients for 1 and 2 months were unable to show reduction in HbA1c and blood glucose concentrations [32,33], while in other long-term trials HbA1c and glucose levels were significantly declined [26,34]. Parallel to these trials our patients experienced no change in their FBS levels at the end of the second week. This finding again strengthens our hypothesis that carvedilol acquires at least several months to reveal its metabolic properties. It should not be forgotten that finding an effective drug that could decrease the oxidative stress of diabetes in short-term treatment is much better than that in the long-term. In fact, this has been well established in previous studies [35–39]. Further case-control studies may clarify this conjecture.

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Highlights

- Oxidative stress is a situation in which the amount of reactive oxygen species (ROSs) exceeds the levels of neutralizing substances referred to as antioxidants.
- Type II diabetes is associated with oxidative stress and there is compelling biochemic evidence that suggests ROSs may play a role in the pathogenesis of type II diabetes.
- Several *in vitro* and animal studies have shown promising evidence with regards to the antioxidant effect of carvedilol in oxidatively stressed models.
- Clinical trials in this field are scarce and controversial, and as yet there are no clinical trials considering diabetes and the antioxidant effect of carvedilol in short-term therapy.
- The present results indicate no improvement in oxidative stress status and no change in fasting glucose levels in diabetic patients treated with carvedilol for 14 days.
- Considering previous trials, it seems that carvedilol exerts its antioxidant effects in long-term treatment; approximately 3 months. Interestingly, the same scenario occurs with regards to fasting blood sugar levels.
- This finding strengthens the hypothesis that carvedilol requires at least several months to reveal its metabolic properties. Further case-control studies may clarify this conjecture.

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