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Original Article

The effect of anti-diabetic agents on biochemical changes of diabetic rats

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Abstract

The present study was performed to investigate the effect of vitamin E as an antioxidant and selenium as a potent insulin-mimetic agent in diabetic rats. Also, to study the ability of these treatments to ameliorate some of the biochemical changes that are worsened with the development of diabetes, such as; serum glucose, blood malondialdehyde (MDA), triglycerides, total cholesterol and β_2-micoglobulin. Experimental diabetes was induced in male rats by intravenous injection of streptozotocin (50mg/kg). Two weeks after the overt of diabetes, rats were divided into groups each of 10 animals. Group1 received normal saline. Group2 received vitamin E acetate (40mg/kg) every other day by I.P. injection for 4 weeks. Group 3 received sodium selenate (1.89 mg/kg) every day by I.P. injection for 18 days. Group 4 non-diabetic control rats received normal saline. Our results revealed that diabetic rats showed a significant increase in serum glucose, blood MDA levels, plasma levels of triglycerides, total cholesterol and β_2-micoglobulin. Treatment of diabetic rats with either vitamin E or sodium selenate produced a significant lowering in serum glucose level. Also, they produced a significant reduction in blood MDA level, plasma triglycerides, total cholesterol and β_2-micoglobulin.

Key words: Vitamin E, sodium selenate, diabetes.

Introduction

Diabetes mellitus is defined as a chronic metabolic disorder of carbohydrate metabolism characterized by hyperglycemia and glycosuria due to deficiency of insulin resulting from either insufficient supply or diminished effectiveness.

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Long standing diabetes results in complications such as microangiopathy, retinopathy, nephropathy and neuropathy [1]. Also, it has been found that elevated extra-and intracellular glucose concentrations result in an oxidative stress [2]. Oxidative stress has been found to be mainly due to an increased production of free radical and a sharp reduction of antioxidant defense [3]. It has been reported that vitamin E content in tissue decreased in diabetic animals [4]. In a recent study, it has been reported that the defective endothelium-dependant relaxation of aorta of diabetic rats was prevented by dietary supplementation of vitamin E [5]. Vitamin E treatment also normalizes glomerular dysfunction such as increased glomerular filtration rate and ameliorates the increased albuminuria caused by diabetes [6]. Selenium is a trace element in nature. Several studies demonstrated that selenium had insulin like action [7]. In addition, selenium has been shown to restore liver glycogen and glucose-6-phosphate activity to near control values in diabetic mice [8]. Furthermore, it has significantly corrected renal hyper-filtration and reduced the number and severity of glomerular lesions in diabetic rats [9].

The aim of this study was to investigate the effect of vitamin E as an antioxidant and selenium as a potent insulin-mimetic agent in diabetic rats. And to study their abilities to ameliorate some of the biochemical changes such as serum glucose, blood malondialdehyde, plasma triglycerides, total cholesterol and β_2-microglobulin levels.

Materials and methods

Induction of Diabetes: Adult male Spraque Dawley rats weighing 200-300 g were used in this study. Rats were anesthetized by inhalation using halothane. Severe diabetes was induced as previously described [10] by intravenous tail-vein injection of streptozotocin (50 mg/kg in 0.1M citrate buffer, pH 4.5). Two days after streptozotocin (STZ) administration, diabetes was

confirmed by the presence of glycosuria using B-M glucotur strips. Two weeks after induction of diabetes, blood samples were taken for determination of non-fasting blood sugar. Rats with serum glucose level more than 400 mg/dl were used in this study.

Treatment Schedule: Two weeks after the induction of diabetes, diabetic rats were randomly divided into groups each of 10 rats according to the following Schedule: Control diabetic rats received normal saline (Group 1). Diabetic rats received vitamin E acetate (40 mg/kg) every other day diluted with sunflower oil and taken by I.P. injection for 4 weeks (Group 2). Diabetic rats received sodium selenate (1.89 mg/kg) every day dissolved in water and given by I.P. injection for 18 days (Group 3). There was a group of normal rats received only normal saline (Group 4).

Determination of glucose: The rat was anesthetized with halothan inhalation. Blood was collected from the orbital sinus by using fine Pasteur pipette. The blood was transferred to a centrifuge tube and left for 10 minutes. The tube was then centrifuged for 10 minutes at 4000 r.p.m. and serum was separated. Serum glucose was measured with Beckman glucose analyzer 2.

Determination of malondialdehyde (MDA): Determination of MDA was taken as an index of lipid peroxidation process. MDA was determined by the method of Stocks and Donnandy [11]. This method depends on the fact that MDA can react with thiobarbituric acid to form a colored complex, which can be measured calorimetrically.

Determination of triglycerides and total cholesterol: Triglycerides were estimated by the method described by Fredrickson and his colleagues [12], using Stanbio Triglycerides Liquicolor Enzymatic Kit. Total cholesterol was determined enzymatically according to the method of Allain and Coworkers [13] using Stanbio Cholestrol Liquicolor Kit.

Determination of β_2-micoglobulin: β_2-microglobulin was estimated by enzyme-linked immunosorbent assay [14]. Samples were allowed to react first with immobilized (on micro titer wells) mouse monoclonal anti- β_2-microglobulin antibody for 30 minutes at 37°C. Sheep anti-β_2-microglobulin conjugate was then added for 30 minutes at 37°C. The wells were washed with distilled water and 3,5`,5 tetramethyl benzidin (TMB) reagent was added for 20 minutes at room temperature. After adding the stopping solution, the absorbance was measured with spectrophotometer at 450 nm.

Statistical analysis: Data were expressed as mean ± standard error of the mean (S.E.M.). Statistical analysis was carried out using one way analysis of variance (ANOVA) followed by Tukey-Kramer multiple comparisons test [15]. Also paired Student's t-test was used to compare after-treatment values with their respective before-treatment values. In addition, linear regression analysis for the best fitting line of all standard points was constructed [15].

Results

Our results showed that the data obtained from control non-diabetic rats are within the range of the normal values. Streptozotocin induced diabetes in rats produced a significant increase in serum glucose levels of about 3-5 folds of the control non-diabetic values as shown in table 1. Treatment of diabetic rats with vitamin E showed a significant decrease in serum glucose level when compared either with their initial values or with control diabetic values but still significantly different from control non diabetic values. Diabetic rats treated with selenium produced a significant decrease in serum glucose levels when compared with their initial values and also when compared with the control diabetic values. On the other hand, there was no significant difference when compared with control non-diabetic value.

Results in table 2, showed that diabetic rats produced a significant increase in blood MDA levels to about 3-4 folds when compared with the control non-diabetic values. Vitamin E treatment produced a significant lowering effect on blood MDA to near the normal levels, while treatment with selenium produced a significant decrease in blood MDA levels when compared with their initial and control diabetic values. Diabetic rats also produced a significant increase in plasma triglycerides levels to about 2-3 folds and a significant increase in plasma total cholesterol and β_2-micoglobulin levels than that of the control non-diabetic values. Diabetic rats treated with either vitamin E or selenium produced a significant reduction in plasma triglycerides, total cholesterol and β_2-microglobulin levels when compared with their initial values or with the control diabetic values table 3,4,5.

Table 1. Effect of vitamin E and selenium treatment on plasma glucose level in diabetic rats

Treatment	*Serum glucose level (mg/dl)*		
	Before treatment	After treatment	% change
Control (saline)	121.5±5.67	115.67±4.49	- 4.79
Control diabetic (saline)	485.83±17.5	561.5±16.29	+ 15
Vitamin E (40 mg/kg)	506.67±12.39	359.17±35.4	- 29.11
Selenium (1.89 mg/kg)	485.33±19.44	186.5±6.72	- 61.57

Table 2. Effect of vitamin E and selenium treatment on plasma malondialdhyde level in diabetic rats

Treatment	*Blood malondialdhyde (umol/ml packed cell)*		
	Before treatment	After treatment	% change
Control (saline)	2.43±0.52	2.24±0.57	- 7.818
Control diabetic (saline)	7.01±0.67	9.62±1.049	+ 8.7
Vitamin E (40 mg/kg)	7.06±0.48	2.33±0.48	- 66.67
Selenium (1.89 mg/kg)	6.54±0.41	3.69±0.22	- 43.57

Table 3. Effect of vitamin E and selenium treatment on plasma triglycerides level in diabetic rats

Treatment	*Plasma triglycerides level (mg/dl)*		
	Before treatment	After treatment	% change
Control (saline)	90.23±6.94	99.98±5.9	+ 10.8
Control diabetic (saline)	208.94±25.63	382±23.11	+ 82.8
Vitamin E (40 mg/kg)	204.11±12.95	89.85±7.36	- 55.97
Selenium (1.89 mg/kg)	276.23±22.29	98.04±5.11	- 64.5

Table 4. Effect of vitamin E and selenium treatment on plasma total cholesterol level in diabetic rats

Treatment	*Plasma total cholesterol level (mg/dl)*		
	Before treatment	After treatment	% change
Control (saline)	55.18 ±1.75	63.09± 4.93	+ 14.3
Control diabetic (saline)	113.61 ±7.29	130.98 ±10.4	+ 15.28
Vitamin E (40 mg/kg)	111.83 ±6.65	80.39± 7.55	- 28.11
Selenium (1.89 mg/kg)	104.76± 5.24	62.62 ±3.76	- 40.2

Table 5. Effect of vitamin E and selenium treatment on plasma β_2 - microglobulin level in diabetic rats

Treatment	*Plasma β_2 - microglobulin level (mg/dl)*		
	Before treatment	After treatment	% change
Control (saline)	5.01 ±0.59	5.327± 0.67	+ 6.33
Control diabetic (saline)	10.97 ±1.41	11.66 ±0.91	+ 6.29
Vitamin E (40 mg/kg)	9.7 ±0.72	6.14± 0.29	- 36.7
Selenium (1.89 mg/kg)	9.76± 0.76	6.48 ±0.16	- 33.57

Discussion

Our results showed that diabetic rats had marked elevated levels of MDA, triglycerides and total cholesterol when compared with control non-diabetic or initial values. Increased blood MDA level may be due to increased plasma lipid peroxide level with the duration of disease and with the development of complication [16]. Hyper-glyceridaemia may be a consequence of defective removal of triglycerides rich lipoproteins from the circulation [17]. Oxidative stress would cause hyper-cholesterolemia through reduction of bile acid synthesis or through reduction in the activity of hepatic hydroxy methyl glutaryl reductase, a key enzyme in cholesterol metabolism. Diabetic rats also exhibited a significant increase in plasma β_2-microglobulin level. Diabetic nephropathy is usually characterized by glomerular dysfunction as well as tubular damage [18]. Previous studies showed a marked increase in plasma β_2-microglobulin level in diabetic patients in comparison with non-diabetic controls [19]. Also, there was a marked increase in urinary β_2-microglobulin level in diabetic rats [20].

Concerning the effect of vitamin E treatment on serum glucose level, our data showed a significant decrease when compared with the initial diabetic and the control diabetic values. Previous studies showed that vitamin E has a positive effect on basal and arginine induced insulin secretion in the pancreatic β-cells that were not destroyed after STZ-injection but, vitamin E did not restore glucose-induced insulin secretion that had been abolished by the diabetic state [21]. Diabetic rats treated with vitamin E showed a significant reduction in blood MDA levels in comparison with the initial diabetic and

control diabetic values. This efficacy due to the powerful antioxidant properties of vitamin E as it is effective as a major peroxyl radical scavenger of bio-membranes and low density lipoproteins, so it inhibits the propagation of lipid peroxidation process [22]. Our results revealed that Vitamin E produced a significant reduction in plasma levels of triglycerides and total cholesterol. This efficacy may be because vitamin E inhibits lipid peroxides and increases low molecular weight triglycerides. Also it increases the total hepatic triglycerides [23]. The effect of vitamin E treatment on plasma β_2-microglobulin showed marked reduction when compared with the initial diabetic and control diabetic values. Vitamin E can ameliorate early diabetic renal injury via inhibiting protein kinase C activity that may prevent or delay the development of diabetic nephropathy [24].

Treatment of diabetic rats with sodium selenate showed a significant reduction in serum glucose level to near normal values. Selenium has been reported to exhibit an insulin mimetic action through its action as a potent stimulator of tyrosyl phosphorylation, it increases the phosphorylation of proteins identified in the insulin cascade and could also affect overall phsphorylation in the cell [25]. Also, selenate affects glucose homeostasis and liver metabolism in diabetic rats and this raises the possibility that selenate exerts non-specific actions on glucose metabolism [7]. Selenium also produced a significant reduction in blood MDA level in diabetic rats. Selenium may function via glutathione peroxide, which has an antioxidant activity since it catalyses the conversion of lipid hydroperoxides to stable non-radical (alcohol), so it is hypothesized that selenium could prevent oxidative damage [26]. Treatment of diabetic rats with selenium produced a significant reduction in plasma levels of triglycerides and total cholesterol. This may be attributed to its insulin mimetic action that increases lipoprotein lipase activity via Ca^{++} mobilization linked to a rapid increase in inositol triphosphate content [27]. Also selenium has the ability to restore the level of hydroxymethyl glutaryl coenzyme A (HMG-CoA) reductase activity, that it can normalize the alteration in cholesterol metabolism that occurs in diabetes [28]. Selenium also produced a significant reduction in plasma β_2-microglobulin level. Previous studies stated that selenium supplementation to diabetic rats prevents, not only oxidative stress, but also renal structural injury thus reduced plasma β_2-microglobulin level [29].

In conclusion vitamin E is a powerful antioxidant that ameliorated most of the biochemical changes induced by oxidative stress, one of the major reasons leading to diabetic complication. Selenium possessed potent insulin mimetic effects manifested by a good glycemic control and a reduction in the increased oxidative stress in diabetic rats.

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