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In vivo treatment with stobadine prevents lipid peroxidation, protein glycation and calcium overload but does not ameliorate Ca^{2+}-ATPase activity in heart and liver of streptozotocin-diabetic rats: comparison with vitamin E

The ADIC (Antioxidants in Diabetes-Induced Complications) Study Group
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Abstract

Hyperglycemia leads to excess production of reactive oxygen species (ROS), lipid peroxidation and protein glycation that may impair cellular calcium homeostasis and results in calcium sequestration and dysfunction in diabetic tissues. Stobadine (ST) is a pyridoindole antioxidant has been postulated as a new cardio- and neuroprotectant. This study was undertaken to test the hypothesis that the treatment with ST inhibits calcium accumulation, reduces lipid peroxidation and protein glycation and can change Ca^{2+},Mg^{2+}-ATPase activity in diabetic animals. The effects of vitamin E treatment were also evaluated and compared with the effects of combined treatment with ST. Diabetes was induced by streptozotocin (STZ, 55 mg/kg i.p.). Some of diabetic rats and their age-matched controls were treated orally with a low dose of ST (24.7 mg/kg/day), vitamin E (400–500 IU/kg/day) or ST plus vitamin E for 10 weeks. ST and vitamin E separately produced, in a similar degree, reduction in diabetes-induced hyperglycemia. Each antioxidant alone significantly lowered the levels of plasma lipid peroxidation, cardiac and hepatic protein glycation in diabetic rats but vitamin E treatment was found to be more effective than ST treatment alone. Diabetes-induced increase in plasma triacylglycerol levels was not significantly altered by vitamin E treatment but markedly reduced by ST alone. The treatment with each antioxidant completely prevented calcium accumulation in diabetic heart and liver. Microsomal Ca^{2+},Mg^{2+}-ATPase activity significantly decreased in both tissues of untreated diabetic rats. ST alone significantly increased microsomal Ca^{2+},Mg^{2+}-ATPase activity in the heart of normal rats. However, neither treatment with ST nor vitamin E alone, nor their combination did change cardiac Ca^{2+},Mg^{2+}-ATPase activity in diabetic heart. In normal rats, neither antioxidant had a significant effect on hepatic Ca^{2+},Mg^{2+}-ATPase activity. Hepatic Ca^{2+},Mg^{2+}-ATPase activity of diabetic rats was not changed by single treatment with ST, while vitamin E alone completely prevented diabetes-induced inhibition in microsomal Ca^{2+},Mg^{2+}-ATPase activity in liver. Combined treatment with ST and vitamin E provided more benefits in the reduction of hyperglycemia and lipid peroxidation in diabetic animals. This study describes potential mechanisms on cellular effects of ST in the presence of diabetes-induced hyperglycemia that may delay or inhibit the development of diabetic complications. The use of ST together with vitamin E can better control hyperglycemia-induced oxidative stress.
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Keywords: Streptozotocin-diabetes; Stobadine; Vitamin E; Ca^{2+}-ATPase; Protein glycation; Lipid peroxidation

1. Introduction

A number of mechanisms, including hyperglycemia, glycosylation of proteins and oxidative stress, have been proposed to contribute to the pathogenesis of cellular

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dysfunction leading to cardiovascular, hepatic and other complications of diabetes [1,2]. Hyperglycemia has been shown to generate reactive oxygen species (ROS) such as superoxide radicals from autooxidation of glucose, and resultant increase in cellular lipid peroxidation in diabetic patients and animals [3,4]. Excess production of ROS may lead to cellular injury through nonspecific modification and disruption of proteins, phospholipids and nucleic acids [5]. Critical sites of ROS attack are the cell membrane and the membranes of intracellular organelles. The disruptive effect of ROS involves membrane lipid peroxidation and membrane protein modification, which may produce alterations in the membrane structure and function including fluidity, permeability, enzyme activity, ion channels and transport, and receptor proteins. Data obtained from animals and patients with diabetes showed that abnormal intracellular calcium metabolism is widespread in tissues [6], and intracellular calcium levels are increased in many tissues including heart [7–10]. Similarly, hepatocyte calcium sequestration and turnover are also increased in both acute and chronic experimentally induced diabetes [11,12]. The activities of membrane ATPases (i.e. Ca^{2+},Mg^{2+}-ATPase and Na^{+},K^{+}-ATPase) has been shown to be abnormal in diabetic state. Decreased Ca^{2+}-ATPase activity has been reported in diabetic heart [7,8,10]. In contrast, streptozotocin (STZ)-induced diabetes led to an increase in hepatic plasma membrane Ca^{2+}-ATPase activity of rats [14]. The studies imply that the nature of the alterations is tissue-specific and may depend on the level of blood glucose or insulin, or both. Membrane Ca^{2+},Mg^{2+}-ATPase is responsible for the fine-tuning of intracellular calcium and the Na^{+}–Ca^{2+} exchanger for rapid ejection of the excess calcium that has entered the cell [15]. Insufficient Ca^{2+},Mg^{2+}-ATPase activity has been suggested as contributing factor in the development of diabetic cardiomyopathy [7,8]. The effects of ROS on the enzyme may be very specific and may include selective alterations of its active sites resulting in inhibition of its activity [16–19].

Supplementation with antioxidants has been shown to decrease oxidative stress and complications in animal models of diabetes [20,21] and in diabetic patients [22]. Diabetes-induced defects in the homeostasis and the transport of intracellular calcium has been shown to decrease or recover by the treatment of diabetic animals with some antioxidant compounds, such as *N*-acetyl cysteine, beta-carotene, vitamin E, trolox C and pyridoxine [19,23,24]. Stobadine (ST), (−)-*cis*-2,8-dimethyl-2,3,4,4a,5,9b-hexa-hydro-1*H*-pyrido[4,3-b] indole, was shown to be able to scavenge hydroxyl, alkoxyl and peroxyl radicals, weakly superoxide, and to quench singlet oxygen, to repair oxidized amino acids and to preserve oxidation of SH groups by one-electron donation [25]. It was demonstrated that in free radical generating systems in vitro, ST and some other lipophilic and water-soluble antioxidants, including butylated hydroxytoluene, lipoic acid and vitamin E, are able to decrease the effects of oxidative stress on tissue Ca^{2+}-ATPase activity and calcium accumulation [16,18,24,26]. We recently showed that the treatment of diabetic rats with a low dose of ST attenuates tissue markers of oxidative stress and other diabetes-induced abnormalities in brain [27], heart [28] and aorta [29]. Since the question of the effect of ST on calcium homeostasis in diabetes mellitus has not yet been addressed, the main purpose of the present study was to evaluate the effects of ST on cardiac and hepatic calcium metabolism in STZ-diabetic rats. For this purpose, the amount of calcium and the activity of Ca^{2+}-ATPase in heart and liver microsomes of normal and STZ-diabetic rats, treated or untreated with ST, were determined. Secondly, we compared the effect of ST treatment alone with that of vitamin E. Since different antioxidant compounds may act synergistically and some combinations may be more effective than any one compound alone, the effects of ST plus vitamin E treatment were also evaluated and compared with the effects of treatment with each agent alone. The effects of ST and vitamin E on plasma triacylglycerol levels, lipid peroxidation and tissue protein glycation were also measured to elucidate the underlying mechanisms.

2. Materials and methods

2.1. Induction of diabetes and treatment protocols

Male Wistar rats, body weight 250–300 g, were fed a standard rat chow diet and had access to water ad libitum. Diabetes was induced by a single intraperitoneal injection of STZ (55 mg/kg body weight) to animals fasted overnight. Diabetes was verified 48 h later by measuring tail vein blood glucose, and the rats with blood glucose of 300 mg/dl or more were considered diabetic. Two days after injection of STZ or vehicle, rats were divided into the following groups: (1) untreated diabetic rats ($n=12$); (2) diabetic rats treated with ST (24.7 mg/kg/day, orally) ($n=11$); (3) diabetic rats treated with vitamin E (alpha-tocopheryl acetate, 400–500 IU/kg/day, orally) ($n=11$); (4) diabetic rats treated with both ST and vitamin E, as given in protocols 2 and 3 ($n=10$); (5) untreated control rats ($n=9$); (6) control rats treated with ST as given in protocol 2 ($n=5$); (7) control rats treated with vitamin E, as given in protocol 3 ($n=5$); (8) control rats treated with ST plus vitamin E as given in protocol 4 ($n=5$). The dose regimen of ST or vitamin E was chosen according to some previous studies [20,25]. The animals were treated for a period of 10 weeks beginning 48 h after either vehicle or STZ injection. "The principles of laboratory animal care" (NIH publication no. 85-23, revised 1985) were observed.

2.2. Measurement of blood glucose, triacylglycerol and thiobarbituric acid reactive substances (TBARS)

Blood glucose and triacylglycerol concentrations were measured by Accutrend® GCT meter (Roche Diagnostics).

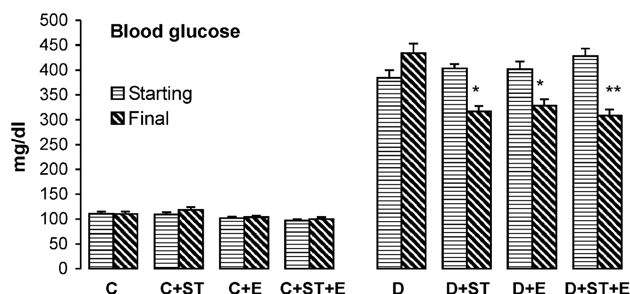

Fig. 1. Blood glucose levels of control (C) and diabetic (D) animals untreated or treated with stobadine (ST), vitamin E (E) or stobadine plus vitamin E (ST + E). Data are reported as means ± S.E. The blood glucose concentrations of all diabetic animals were significantly different from the control animals untreated or treated with antioxidants. Start: 2 days later of STZ injection. Final: at the end of the treatment period. * $P < 0.05$, ** $P < 0.01$ vs. starting within group.

TBARS, end products of lipid peroxidation, were measured fluorometrically in plasma [30].

2.3. Membrane-enriched microsome preparation

Heart and liver tissues were weighed and homogenised in six volumes of freshly prepared buffer A containing: 0.3 mol/l sucrose, 10 mmol/l HEPES–HCl pH 7.4 and 2 mmol/l dithiothreitol. A Teflon/glass homogeniser (Omni mixer homogeniser model 18074, Omni Int., CT, USA) was used for homogenisation of tissues. The homogenates were centrifuged at $85,000 \times g$ for 75 min, the supernatant discarded and the pellets were resuspended in the original volume of buffer A containing 0.6 mol/l KCl (using four strokes) and centrifuged again at $85,000 \times g$ for 75 min. The pellets were then resuspended in the original volume of buffer A. After centrifugation at $85,000 \times g$ for 75 min (Beckman L 7, Beckman Instruments Inc., Fullerton, CA), the pellets were suspended in buffer A (using four strokes) at a protein concentration of 2–7 mg/ml. Samples were kept frozen at − 60 °C until further use. Isolation procedure was performed at + 4 °C [31].

2.4. Determination of Ca^{2+}-ATPase activity

Ca^{2+}-ATPase activity was measured spectrophotometrically (Pye Unicam SP8-100 UV spectrophotometer, PYE Unicam Ltd., Cambridge, England), as described previously [32]. The incubation medium contained: 120 mmol/l KCl, 60 mmol/l HEPES, pH 7.0 (at 37 °C), 1 mmol/l $MgCl_2$, 0.5 mmol/l K_2-ATP, 0.2 mmol/l NADH, 0.5 mmol/l PEPA, 1 IU pyruvate kinase, 1 IU lactate dehydrogenase/ml and 500 μmol/l EGTA. After preincubation of the assay medium (total volume of 1 ml) for 4 min at 37 °C, 75-μg ATPase was added to the medium. After 2 min, the reaction was started by addition of 600 μmol/l $CaCl_2$. Determination of ATPase activity was followed by continuous measurement of absorbance at 365 nm.

2.5. Determination of tissue calcium levels and protein glycation

Tissue calcium levels were analysed by atomic absorption spectrophotometry (Perkin Elmer 2380) according to the wet-ashing procedure with nitric acid.

Protein glycation in microsomal fractions was measured as described previously [33].

2.6. Drugs and statistical analysis

All chemicals except ST were purchased from Sigma Chemical (St. Louis, MO, USA). ST dipalmitate was obtained from the Slovak Academy of Sciences. Data are expressed as mean ± S.E. They were first subjected to Bartlett's test for homogeneity of variances and were given a log transformation if necessary. One-way analysis of variance was then performed, followed by the Student–Neuman–Keuls test to estimate the significance of differences for individual between-group comparisons. For the statistical evaluation of starting and final blood glucose concentrations within groups, Student's *t* test was used.

3. Results

The final blood glucose concentrations of untreated diabetic rats were about four times higher than in normal control rats (Fig. 1). A treatment with each agent alone produced a significant fall in blood glucose levels of diabetic animals. The magnitude of the blood glucose lowering effect of ST treatment was found to be comparable to the effects of vitamin E treatment. Combination of ST and vitamin E provided some further benefit on blood glucose control; nevertheless, at the end of the treatments, diabetic rats were still hyperglycaemic when compared with normal control rats (Fig. 1).

Plasma triacylglycerol concentrations significantly increased in untreated diabetic rats (Fig. 2). Single treatment with ST resulted in a marked decrease in plasma triacylgly-

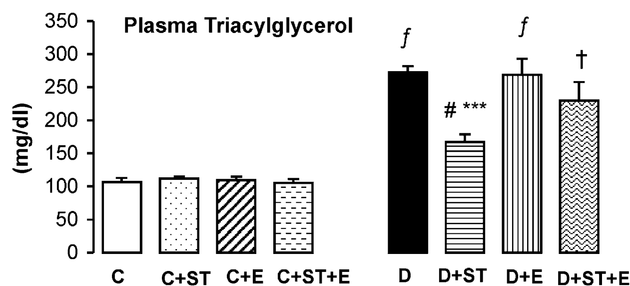

Fig. 2. Plasma triacylglycerol levels of control (C) and diabetic (D) animals untreated or treated with stobadine (ST), vitamin E (E) or stobadine plus vitamin E (ST + E). Data are reported as means ± S.E. # $P < 0.05$, † $P < 0.01$, f $P < 0.001$ vs. untreated-control (C) animals. ** $P < 0.01$, *** $P < 0.001$ vs. untreated-diabetic (D) animals.

cerols while vitamin E alone was unable to induce a significant reduction in elevated triacylglycerols of diabetic rats (Fig. 2). Combined treatment has no further effect on the decreasing of triacylglycerol levels of diabetic animals.

The levels of TBARS, end products of lipid peroxidation, were markedly elevated in plasma of diabetic rats compared to those in controls (Fig. 3). This increase was partially prevented by treatment with either ST or vitamin E alone. In the prevention of plasma lipid peroxidation, the combined therapy was significantly more effective than treatment with a single agent, and it provided almost normalised plasma TBARS in diabetic animals (Fig. 3).

In normal control rats, treatment with each antioxidant studied, as well as their combination, led to a significant reduction of cardiac protein glycation. In these animals, hepatic protein glycation was also inhibited by ST alone, yet not by vitamin E. Glycation of proteins significantly increased in heart and liver of untreated diabetic rats (Fig. 4). In heart of diabetic rats, an increase in protein glycation was significantly but not completely prevented by vitamin E treatment; while this vitamin completely prevented diabetes-induced changes in glycation of hepatic proteins. ST, when given alone, was also effective in the prevention of protein glycation increase in both diabetic heart and liver; however, in comparison with vitamin E, the inhibitory effect of ST was moderate. When compared with the effect of viatamin E treatment alone, the combination of the two antioxidants did not provide an additional benefit on either cardiac or hepatic protein glycation of diabetic rats (Fig. 4).

Total calcium levels were markedly increased in heart and liver of untreated diabetic rats (Fig. 5). In the heart, treatment with either ST or vitamin E resulted in a significant decrease in calcium levels in both nondiabetic control and diabetic rats. In the treatment with vitamin E alone or two agents together, the calcium levels of the diabetic heart were found to be lower than those of normal control animals. In the liver, neither ST nor vitamin E treatment did significantly change calcium levels of normal control rats, but in diabetic rats calcium overload was completely prevented (Fig. 5).

Microsomal Ca^{2+}-ATPase activity significantly decreased in heart and liver of untreated diabetic rats (Fig. 6).

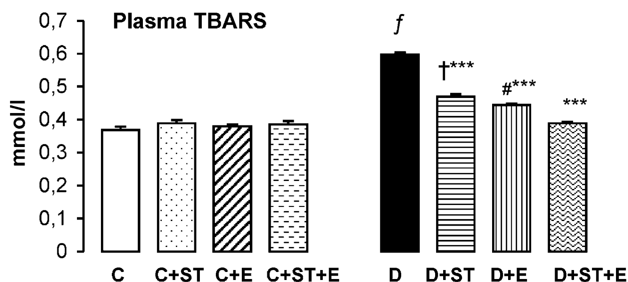

Fig. 3. Effects of stobadine (ST) and vitamin E (E) treatments on plasma lipid peroxidation levels (Thiobarbituric acid reactive substances (TBARS) of the animals. Data are reported as means ± S.E. $^{\#}P<0.05$, $^{\dagger}P<0.01$, $^{f}P<0.001$ vs. untreated-control (C) animals. $^{**}P<0.01$, $^{***}P<0.001$ vs. untreated-diabetic (D) animals.

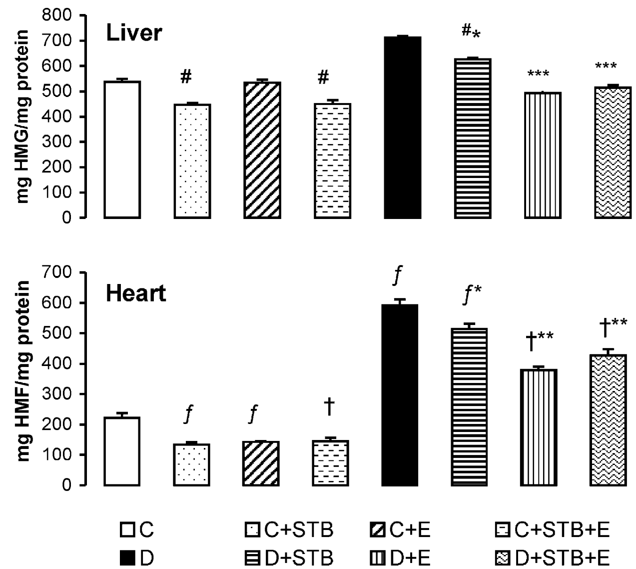

Fig. 4. Effects of stobadine (ST) and vitamin E (E) treatments on the glycation of proteins in liver and heart of the animals. Data are reported as means ± S.E. $^{\#}P<0.05$, $^{\dagger}P<0.01$, $^{f}P<0.001$ vs. untreated-control (C) animals. $^{*}P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$ vs. untreated-diabetic (D) animals.

The inhibitory effect of diabetes on Ca^{2+}-ATPase activity was observed to be higher in heart than that in liver. ST alone or its combination with vitamin E significantly increased microsomal Ca^{2+}-ATPase activity in the heart of normal rats. However, neither treatment with ST nor vitamin E alone, nor their combination did change cardiac Ca^{2+}-ATPase activity in diabetic heart. In normal rats, neither antioxidant had a significant effect on hepatic Ca^{2+}-ATPase activity. Hepatic Ca^{2+}-ATPase activity of diabetic rats was

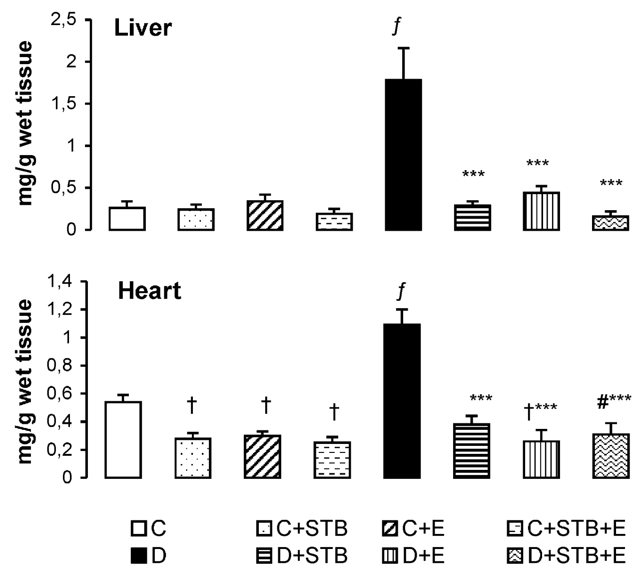

Fig. 5. Tissue calcium concentrations in control (C) and diabetic (D) animals untreated or treated with stobadine (ST), vitamin E (E) or stobadine plus vitamin E (ST + E). Data are reported as means ± S.E. $^{\#}P<0.05$, $^{\dagger}P<0.01$, $^{f}P<0.001$ vs. untreated-control (C) animals. $^{*}P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$ vs. untreated-diabetic (D) animals.

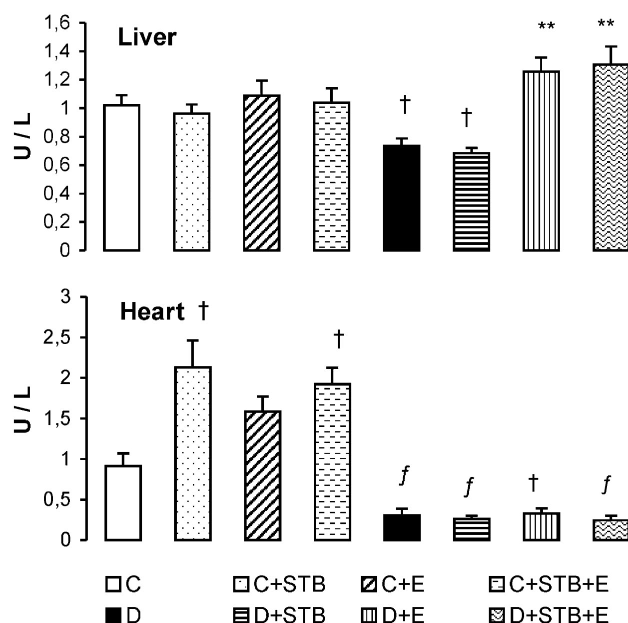

Fig. 6. Microsomal Ca^{2+}-ATPase activity in control (C) and diabetic (D) animals untreated- or treated with stobadine (ST), vitamin E (E) or stobadine plus vitamin E (ST + E). Data are reported as means ± S.E. $^{\#}P<0.05$, $^{\dagger}P<0.01$, $^{f}P<0.001$ vs. untreated-control (C) animals. $^{*}P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$ vs. untreated-diabetic (D) animals.

not changed by the treatment with single ST, while vitamin E alone completely prevented diabetes-induced inhibition in microsomal Ca^{2+}-ATPase activity in liver (Fig. 6).

4. Discussion

Abundant information is now available on changes in plasma membrane and subcellular organelles, which are responsible for the impaired intracellular calcium homeostasis in diabetic cardiomyopathy and other complications of diabetes mellitus [6,7,9,10]. Some of these include abnormalities in the following variables: calcium binding, influx of calcium through the L-type calcium channels, Na^{+},K^{+}-ATPase activity, Na^{+}–Ca^{2+} exchange, H^{+}–Na^{+} exchange and Ca^{2+},Mg^{2+}-ATPase activity etc. The Ca^{2+}-ATPase is the major active calcium transport protein responsible for the maintenance of normal intracellular calcium levels in a variety of cell types. Maintenance of the cation gradient by Ca^{2+}-ATPase is of fundamental importance in the control of hydration, volume, nutrient uptake and fluidity of cells, and is also essential for the contractility and excitability properties of muscles [6,7]. In the present study, microsomal Ca^{2+},Mg^{2+}-ATPase activity was depressed by STZ-diabetes in heart and liver of rats. This event was accompanied to increased total calcium levels. Our findings are in agreement with previous observations showing that the membrane abnormalities in Na^{+},K^{+}-ATPase, Na^{+}–Ca^{2+} exchange and Ca^{2+}-pump activities led to the occurrence of intracellular calcium overload in experimental rat models of diabetes [7,10,13,34]. Depressed myofibrillar and sarcolemmal Ca^{2+}-ATPase activity and sarcoplasmic reticulum calcium uptake were reported as important mechanisms responsible for the cardiac dysfunction and cardiomyopathy exhibited by insulin-deficient (Type I) diabetic animals [7,10,13,34].

The increased intracellular concentration of calcium may be explained by the osmotic activity of high glucose (cell shrinkage), demonstrated to activate G protein(s), most likely through a stretch receptor, which in turn stimulates calcium channels inhibitable by verapamil, nifedipine and amlodipine, thus permitting a calcium influx into cardiac myocytes [9]. The increased cardiac affinity for calcium in diabetic rats was also reported to be due to changes in sarcolemmal lipid bilayer composition secondary to diabetes-induced hyperlipidaemia [35]. Diabetes-induced hyperlipidemia and the alterations in membrane phospholipids and fatty acids have been shown to depress membrane-bound enzyme activities, which influence intracellular calcium metabolism resulting in cardiac dysfunction [34]. As indicated before, plasma membrane and the membranes of intracellular organelles are crucial targets of ROS attack. Oxidative insult was found to lead to a decrease in Ca^{2+}-ATPase activity in heart [36,37] or brain microsomes [18]. Decreased membrane fluidity induced by increased oxidative stress has been linked with the aforementioned abnormalities in calcium metabolism [18]. The present study provided once again evidence that there are significant increase in plasma triacylglycerol levels and the peroxidation of lipids in diabetic animals. As it is well known, hyperglycemia results in increased oxidative stress from excessive ROS production from the auto-oxidation of glucose and glycated proteins [2,5,38]. The increased ROS activity initiates peroxidation of lipids and MDA accumulation, which in turn can stimulate glycation of proteins in diabetes [4,38]. Another possibility involves the modification of enzyme molecules either by direct oxidation or by modification mediated by products of lipid peroxidation [39]. A decrease in ATPase enzyme activity in any diabetic tissue could be due to excessive nonenzymatic glycation of the enzyme itself and/or of calmodulin [40]. Ca^{2+}-ATPase was shown to be particularly sensitive to cross-linking and the concomitant decrease in protein rotational mobility due to protein aggregation [36]. In the present study, cardiac and hepatic protein glycation was markedly increased in STZ-diabetic rats; in the light of previous observations, both glycosylation of enzyme proteins and/or inhibition of its activity by lipid peroxidation products seem to be major contributing factors associated with abnormal calcium homeostasis in diabetic animals.

In the present study, we demonstrated for the first time that 10 weeks of ST treatment completely prevented cardiac and hepatic calcium accumulation without a significant amelioration in Ca^{2+}-ATPase activity in STZ-diabetic rats. The magnitude of this effect of ST was parallel with the same effect of vitamin E in both tissues; a combination of ST plus vitamin E did not produce a further effect on total

calcium amount in diabetic tissues. On the other hand, the effect of each antioxidant on microsomal Ca^{2+}-ATPase activity was tissue-specific. In heart of normal control rats, ST treatment alone led to an increase in Ca^{2+}-ATPase activity, but did not produce a significant change in depressed Ca^{2+}-ATPase activity in diabetic rats. In liver, however, ST treatment alone had no significant effect on Ca^{2+}-ATPase activity in either control or diabetic animals. In diabetic animals, vitamin E, although unable to prevent Ca^{2+}-ATPase depression in heart, completely protected hepatic Ca^{2+}-ATPase activity from the destructive effect of STZ-diabetes. In addition, neither ST nor vitamin E did alter hepatic Ca^{2+}-ATPase activity in normal animals.

Previous studies have reported that supplementation with some antioxidants prevents lipid peroxidation, hemoglobin glycosylation and inhibition of Na^{+},K^{+}-ATPase and/or Ca^{2+}-ATPase activity caused by hyperglycemia in various cells [19,23,24]. The exact mechanism(s) of the effects of the both antioxidants on abnormal intracellular calcium regulation of diabetic heart and liver are not fully known. As demonstrated previously, ST prevents the decrease in membrane fluidity and nonspecifically inhibits various excitable channels, and diminishes in a concentration-dependent manner calcium (L-type) inward currents [25] that may account for its preventive effect on calcium accumulation in diabetic tissues. In endoplasmic reticulum membranes of synaptosomes and erythrocytes, ST has been shown to attenuate calcium permeability already increased by a free radical generating system in vitro [16,18]. In this in vitro system, brain Ca^{2+}-ATPase activity was protected by ST, and the efficiency of ST to prevent calcium transport changes was demonstrated to depend on the presence of glutathione and was similar to that of a good chain-breaking antioxidant, butylated hydroxytoluene [18]. In the aforementioned study, in contrast to ST, vitamin E was shown to be less potent to confer the defence against ROS-induced changes in calcium regulation [18]. It has been suggested that inhibition of Ca^{2+}-ATPase is not based on direct protein modifications, but is rather mediated by changes in membrane fluidity and protein–lipid interaction [18]. In the present study, we observed that in vivo treatment with a low dose of ST partially prevented glycation of cardiac and liver proteins and the peroxidation of plasma lipids. These effects of ST treatment alone were less potent than the corresponding effects of vitamin E treatment in diabetic animals. It has been shown that ST inhibits protein glycation and lipid peroxidation [25,41,42]. The inhibitory effect of ST on hyperglycemia-induced lipid peroxidation could be a result of the scavenging of ROS. ST was shown to be able to scavenge hydroxyl, alkoxyl and peroxyl radicals, weakly superoxide, and to quench singlet oxygen, to repair oxidized amino acids and to preserve oxidation of SH groups by one-electron donation [25]. The protein glycation-inhibiting effect of ST has been demonstrated in an experimental in vitro glycation model [42]. Since ST did not affect the covalent binding of glucose, the protective effect of ST has been attributed to its ability to eliminate free radical intermediates of glyco-oxidation reactions, operative after the preceding glycation step [42,43]. ST inhibits glucose-induced chromo- and fluorophore formation, interferes with metal-catalysed oxidation reaction following after the glycation step and limits the damage from glycation-induced processes [43]. Blood-glucose-lowering effect of ST treatment, started 2 days later of STZ injection, might be directly related to its free radical (peroxyl radicals) scavenging properties, which may protect pancreatic beta cells against the STZ toxicity [25].

Vitamin E treatment alone completely prevented protein glycation in diabetic liver, which was parallel with the protection of microsomal Ca^{2+}-ATPase. This implies that impaired Ca^{2+}-ATPase is more likely a consequence of increased protein glycation in diabetic tissue. This phenomenon, however, may be valid for diabetic liver only. In the heart of diabetic animals, the findings suggest that other factors are also involved in the regulation of calcium homeostasis affected by ST and vitamin E. These factors seem unlikely to be directly linked with lipid peroxidation since both antioxidants significantly prevented overproduction of lipid hydroperoxides both in plasma (as observed in this study) and in heart [28] of diabetic animals. In earlier studies, other investigators found that administration of vitamin E in a combination with other antioxidants, *N*-acetyl cysteine, beta-carotene and vitamin C, prevented both diabetes- and galactosemia-induced elevations in oxidative stress, and protected myocardial and retinal Na^{+},K^{+}-ATPase and Ca^{2+}-ATPases against oxidative damage [19,44]. Another report stated that pre-treatment of animals with vitamin E attenuated depression in sarcoplasmic reticulum calcium transport, intracellular calcium accumulation and heart dysfunction in catecholamine-induced cardiomyopathy [26]. Vitamin E can block the glycation of proteins by inhibiting MDA formation [38]. However, some reports suggest that the Ca^{2+}-releasing and/or -retaining effects of alpha-tocopherol may be independent of pro- and/or antioxidant activities [45].

Our study provided the first evidence that a low dose of ST is able to lower blood glucose and triacylglycerol levels and to prevent lipid peroxidation, protein glycation and tissue calcium accumulation in STZ-diabetic rats. The inhibitory effect of ST on calcium overload seems to be dependent largely on its aforementioned beneficial effects but is unlikely to be directly linked with the membrane Ca^{2+}-ATPase activity since ST treatment is unable to prevent Ca^{2+}-ATPase reduction in diabetic heart and liver. The increasing effect of ST on cardiac Ca^{2+}-ATPase activity in normal control rats may be attributed to its lowering effect on calcium amount in this tissue. On comparing the two compounds, vitamin E was found to be more effective than ST in the prevention of lipid peroxidation and protein glycation. These antioxidants provide more benefits only in the prevention of diabetes-induced hyperglycemia and lipid peroxidation when used in combination for the treatment of

diabetes. In addition, ST is a potent inhibitor of plasma triacylglycerol levels. This study describes potential mechanisms on cellular effects of ST in the presence of hyperglycemia that may delay or inhibit the development of diabetic complications. The use of both antioxidants together can provide a better control on hyperglycemia-induced oxidative stress.

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