Research Article [Araştırma Makalesi]

Regulation of Glutathione S-Transferase Mu with type 1 diabetes and its regulation with antioxidants

[Glutatyon S-Transferaz Mu izoziminin Tip 1 diyabet ve antioksidanlar ile düzenlenmesi]

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ABSTRACT

Objective: Increased oxidative stress is now related with the pathogenesis and the chronic complications associated with the disease, diabetes mellitus. While roles of oxidative stress in diabetic complications are widely studied, the molecular mechanisms playing role in the regulations of detoxification enzymes in the presence of antioxidants have not been clearly established because of the complexity of the pathways.

Methods: Diabetes was induced by single dose streptozotocin application and rats were treated with 50mg/kg antioxidants DL-α-lipoic acid (LA), vitamin C (VC) and combination of both for three weeks. Lipid peroxidation, protein oxidation and reduced glutathione levels were measured and Glutathione S-Transferase Mu (GST-Mu) isozyme activity was determined by using enzyme specific substrate. mRNA and protein expressions were evaluated by RT-PCR and western blot methods respectively.

Results: In diabetic animals the products of lipid peroxidation and protein oxidation reactions were all found to be elevated significantly ($p < 0.05$) and supplementing the animals either individually or in combination with two powerful antioxidants restored this effect. GST-Mu activities were also significantly decreased in diabetic group compared to controls and RT-PCR and Western blot analysis results showed that this decrease in activity is regulated at the level of gene expression, as both mRNA and protein expressions were suppressed. All antioxidant treatments did not reverse the effect of diabetes at the gene expression level and interestingly protein expressions were further suppressed indicating a post-translational regulation by antioxidants.

Conclusion: Presence of oxidative stress and the effects of antioxidants in diabetic rats were confirmed and it was found that diabetes leads to GST-Mu to be down regulated at mRNA expression levels. Future post-translational modification studies will help to determine the mechanism or combination of mechanisms underlying the damaging effects of oxidative stress observed in streptozotocin induced diabetic rats.

Key Words: GST-Mu, gene expression, lipid peroxidation, protein carbonylation, diabetes mellitus

Conflict of Interest: The authors declare that they do not have any conflict of interest.

ÖZET

Amaç: Günümüzde artan oksidatif stresin, diyabet hastalığı ile ilişkili kronik komplikasyonlar ve diyabetin patogenezi ile ilişkisi olduğu bilinmektedir. Oksidatif stresin diyabetin komplikasyonlarındaki görevleri çok çalışılmış olmasına rağmen, detoksifikasyon enzimlerinin diyabet ve antioksidan uygulamaları ile moleküler olarak nasıl düzenlendiği, hücre içi yolakların karmaşıklığı nedeniyle açıkça belirlenememiştir.

Yöntemler: Diyabetin hayvansal modeli, sıçanlarda tek doz streptozotocin uygulaması ile oluşturulmuştur. Sıçanlar üç hafta boyunca DL-α-lipoik asit (LA), vitamin C (VC) ve her ikisinin kombinasyonu ile muamele edilmiş, bütün gurupların karaciğer dokularında lipit peroksidasyonu, protein oksidasyonu ve indirge GSH düzeyleri ölçülmüştür. Glutatyon S-Transferaz Mu (GST-Mu) izozim aktivitesi enzime özel substrat kullanılarak, mRNA ve protein ekspresyonu RT-PCR ve Western blot teknikleri ile belirlenmiştir.

Bulgular: Diyabetik hayvanlarda lipid peroksidasyonu ve protein oksidasyonu ürünlerinin istatisitksel olarak yükseldiği ($p < 0.05$) ve LA ve VC gibi iki güçlü antioksidanın tekil veya birlikte uygulamasının bu yükselmeyi normale getirdiği bulunmuştur. GST-Mu enzim aktivitesinin diyabetik gurupta anlamlı ölçüde azaldığı ve bu azalmanın nedeninin, RT-PCR ve Western blot analizleri ile elde edilen düşük mRNA ve protein ekspresyon seviyelerine dayanarak, enzimin gen ekspresyonu düzeyinde gerçekleştiği ortaya çıkarılmıştır. Antioksidan uygulamalarının gen ekspresyon düzeylerini değiştirmediği, fakat protein ekspresyonlarını ilave olarak düşürdüğü bulunmuş, bu durum GST-Mu izoziminin antioksidanlar ile post-translasyonel olarak düzenlenmesi ile açıklanmıştır.

Sonuç: Çalışma ile diyabette oksidatif stresin ve antioksidan uygulamalarının etkileri ortaya çıkarılmış, GST-Mu enziminin diyabet ile aşağı yönde düzenlendiği ve düzenlenmenin gen ekspresyonu düzeyinde gerçekleştiği bulunmuştur. İleri post-translasyonel modifikasyon çalışmaları, streptozotocin ile oluşturulmuş diyabetik sıçanlarda oksidatif stresin detoksifikasyon enzimlerinin düzenlenme mekanizmalarını ortaya çıkaracaktır.

Anahtar Kelimeler: GST-Mu, gen ekspresyonu, lipid peroksidasyonu, protein karbonilasyonu, diabetes mellitus

Çıkar Çatışması: Yazarlar çıkar çatışması bulunmadığını beyan ederler.

Introduction

Oxidative stress is defined as inequality between the productions of reactive oxygen species (ROS) and the organism's antioxidant defense potential. Cells and tissues control the levels of reactive oxygen species with the help of enzymatic and non-enzymatic cellular defense mechanisms. Enzymatic systems include antioxidant enzymes such as catalase (CAT: EC 1.11.1.6), superoxide dismutase (SOD: EC 1.15.1.1) and glutathione peroxidase (GPx: EC 1.11.1.9), whereas non-enzymatic ROS regulation is achieved by scavengers including glutathione (GSH), α-lipoic acid (LA), vitamin C (VC) and α-tocopherol [1,2]. LA and VC are non-enzymatic ROS scavengers which can alleviate intracellular ROS stress. LA and its reduced form dihydrolipoic acid (DHLA) are strong ROS scavengers as a result of their interactions with various radicals and oxidized form of other radicals promoting the antioxidant effects of other antioxidants like VC. Another protective mechanism against oxidative stress is Glutathione S-transferases (GST: EC 2.5.1.18) which catalyze the conjugation of glutathione to a wide range of electrophiles. GSTs also exhibit differential response to a wide variety of chemicals and oxidative stress in the normal and pathophysiological conditions such as diabetes mellitus [3-5]. It has been confirmed that GSTs are also regulated both in vivo and in vitro by reactive oxygen species (ROS) such as superoxides, H_2O_2 and by the products of membrane lipid peroxidation [6,7]. Among the different isoenzymes of GSTs, the cytosolic Mu isoenzyme is predominantly present in the liver and function in the elimination of diverse array of foreign compounds as well as wide variety of products of oxidative stress. Even though, alpha class GST isoenzyme have been shown to possess highest activity towards the lipid peroxidation end product which is 4-hydroxynonenal, GST Mu and Pi also display impressive catalytic efficiency toward this end product [8,9]. Therefore, GST-Mu family is critical in the protection of cells from reactive oxygen species since the total pool of GST-Mu which is quite large in liver cells might contribute to the process of detoxification of the products of the oxidative stress. The aim of this study was to demonstrate how the regulation of the expression of GST-Mu under oxidative stress conditions is taking place. Therefore this study may provide new insights for analyzing cellular mechanism for protection of liver cells against oxidative damage observed in diabetes.

Materials and Methods

Materials

Streptozotocin, DL-6,8-Thioctic acid (a-lipoic acid), ascorbic acid (vitamin C), reduced glutathione (GSH), Bovine serum albumin (BSA), Folin-Ciocalteu Phenol Reagent, tris (hydroxymethyl)aminomethane (Tris), Ethylenediaminetetraacetic acid (EDTA), 5,5'-dithiobis-(2-nitrobenzoic acid (DTNB), Phenylmethylsulfonyfloride (PMSF), Dithiothreitol (DTT), 5-Bromo-4-chloro-3-indolyl phospha-

te (BCIP), Nitro blue tetrazolium (NBT) were purchased from Sigma (St. Louis, MO, USA). All other chemicals used were of the highest analytical grade available, and the buffers were prepared by using sterile distilled water.

Induction of diabetes and animal treatments

Male Wistar rats which were at the same age and weight (200-250gr) were used as experimental animals. All the experimental protocols were performed according to rules of local ethical committee. Animals were fed with a standard diet and diabetes was induced with single injection of streptozotocin (STZ) (55mg/kg) dissolved in 0,05M citrate buffer (pH: 4.5) to the IP cavity of animals. One week after STZ injection, blood glucose levels were checked with Accu-check-go blood glucose analyzer (Roche, Foster City, California, USA) and the animals having blood glucose concentration higher than 200 mg/dl were taken as diabetic. Then, LA (50mg/kg/day) in physiological saline, VC (50mg/kg/day) in physiological saline, and combination of them (50+50 mg/kg/day) were given with daily IP injection until the end of four weeks diabetic growing period. After the diabetes and antioxidants application, experimental test and control groups were as follows: control (n=9), diabetic (n=9), diabetic group given VC (D+VC) (n=12), diabetic group given LA (D+LA) (n=8), diabetic group given combination of VC and LA (D+LA+VC) (n=7). Animals were decapitated after four weeks of diabetes and three weeks of antioxidant treatment and then liver tissues were collected, quick frozen in liquid nitrogen, and kept at -85°C for subsequent biochemical analysis.

Tissue homogenization

Liver tissues were homogenized in 1,15% (w/v) KCl, 5mM EDTA, 0,2mM PMSF, 0,2mM DTT, in 25 mM phosphate buffer, pH 7,4 using teflon-glass homogenizer and homogenates were centrifuged at 1.500g. Supernatants were sampled for the determination of enzyme activities, reduced glutathione (GSH), lipid peroxidation (MDA), protein carbonylation, and for western blot analysis. Protein contents of the homogenates were determined according to Lowry method [10].

Total RNA isolation and RT-PCR studies

Total RNAs were isolated from the liver tissues by guanidine isothiocyanide method[11] and cDNA synthesis by using 1 µg total RNA were carried out by using Revert Aid™ cDNA synthesis kit according to manufacturer protocols (Fermentas; Burlington, Canada). Multiplex RT-PCR was performed in order to amplify GST-Mu mRNA and the internal control gene (β-actin) simultaneously as described previously [12]. The primer pairs used for amplification of cDNAs of GST-Mu were 5′-AGAAGCAGAAGCCAGAGTTC (forward) and 5′-GGGGTGAGGTTGAGGAGATG (reverse) and β-actin were 5′-CCTGCTTGCTGATCCACA (forward) and 5′-CTGACCGAGCGTGGCTAC (reverse).

Western Blot anlaysis

For the western blot analysis of GST-Mu, 20 µg of total cytosolic protein were separated by SDS-PAGE and electroblotted onto PVDF membrane [13]. Then, membranes were incubated with primary antibody solutions of GST-Mu (1:2000- Anti-GST-Mu Rabbit IgG, Abcam: Cambridge, USA), and GAPDH (1:2000- Anti-GAPDH Rabbit IgG, Santa Cruz, USA) for 2 hours with constant shaking. GAPDH was used as internal standard for the normalization. Afterwards, alkaline phosphatase (AP) conjugated secondary antibodies (1:10.000) were applied; proteins were visualized by BCIP/NBT substrate solution. Then, membranes were scanned and the intensities of the bands corresponding to antioxidant enzymes and GAPDH were measured with the aid of Image J software [14].

Activity measurements

GST-Mu Class isozyme activity was determined according to the method of Habig and coworkers [15] with small modifications. Enzyme activity was calculated as the amount of thioether (nmol) formed by 1 mg total protein in one minute by using $0,0085$ µM.cm^{-1} as an extinction coefficient of thioether that is formed by GSTs.

Determination of oxidative biomarkers

GSH determination was carried out based on the oxidation of reduced GSH by 5,5'-dithiobis-(2-nitrobenzoic acid), [DTNB] to produce pale yellow color that gives its maximum absorbance at 412 nm [16]. The results were expressed as nmol GSH per mg protein. MDA concentrations were determined by using extinction coefficient of colored complex as $1,56 \times 10^5$ $(M.cm)^{-1}$ and expressed as nmol MDA for one mg of protein containing supernatant [17]. A spectrophotometric assay [18] was used for the quantification of protein carbonyl groups as a biomarker of oxidative damage. Carbonyl contents of the proteins were calculated by using a molar absorption coefficient of $2,2 \times 10^4$ lt/mol.cm.

Statistical Analysis

Data were given as mean ± standard error of mean (SEM). SPSS 15.0 statistical software (IBM Corporation, Armonk, NY, USA) was used for the calculations of statistical significance between groups that was determined by one-way ANOVA with appropriate post hoc test (Tukey's Honestly Significant Difference). Probability of 0,05 as the level of significance was set in the data analysis and the comparisons.

Results

Biomarkers of oxidative stress: GSH, MDA and protein carbonyl concentrations

Increasing evidence in both experimental and clinical studies suggests that oxidative stress plays a major role in the pathogenesis of diabetes mellitus. We studied the oxidative stress caused by diabetes in liver and determine the extent of oxidative damage by quantifying the oxidation products of the lipids and proteins by measuring the MDA content and protein carbonylation, respectively. As an important oxidative stress marker, GSH concentrations were also determined. Figure 1a demonstrates how GSH levels were modified with diabetes and antioxidant treatments. According to the results, diabetes reduced GSH concentrations below the levels of control groups and the decrease was found statistically significant ($p < 0,05$). Even though, individual LA (α-lipoic acid) and VC (vitamin C) treatments brought the diabetic GSH state toward the control levels, their combination had no significant effect on GSH concentration.

One of the end products of lipid peroxidation reactions is malonedialdehyde (MDA) which was found to be elevated significantly ($p < 0,05$) in diabetic animals. Supplementing the animals with antioxidants either alone or in combination reduced the effect of diabetes and the MDA values reached almost to control values as shown in Figure 1b. Similarly protein carbonyl contents, the indicator of radical induced oxidative protein damage, were found to be higher in diabetics compared to controls. About 30% elevation was observed in diabetes which was also statistically significant ($p < 0,005$). Both LA and VC treatments and their combination reduced the increased protein carbonyl contents observed in diabetes toward the control values, as shown in Figure 1c. The antioxidant treatments either alone or in combination seem to prevent at least some of the consequences of oxidative stress which can promote the development of complications of diabetes mellitus. Table 1 summarizes the overall changes in the reduced glutathione concentrations, and the degree of lipid peroxidation and protein carbonylation in control, diabetic, and the antioxidant supplemented diabetic rat liver tissues.

Regulation of GST-Mu activity in diabetes and the effect of antioxidants

As the activity of GST Mu is related to cellular defense against oxidative stress, regulation of its expression is an important factor in cellular response to a wide variety of oxidants and antioxidants. Previous studies have shown that altered GST isoenzyme expression has been implicated in oxidative stress. In this study GST-Mu isoenzyme was selected as an indicator of defense against oxidative stress. GST-Mu mRNA expressions were determined with multiplex RT-PCR. Figure 2 shows the agarose gel electrophoresis bands of the RT-PCR products obtained from multiplex amplification of GST-Mu and β-actin mRNAs.

After densitometric analysis of the gels with ImageJ software, the ratios of the densities of GST-Mu and β-actin genes were calculated and the results are given as bar diagrams in Figure 4a. According to the results, diabetes caused a significant decrease in mRNA expressions of GST-Mu, and this reduction was statistically significant ($p < 0,05$). Supplementing the rats with either antioxidant
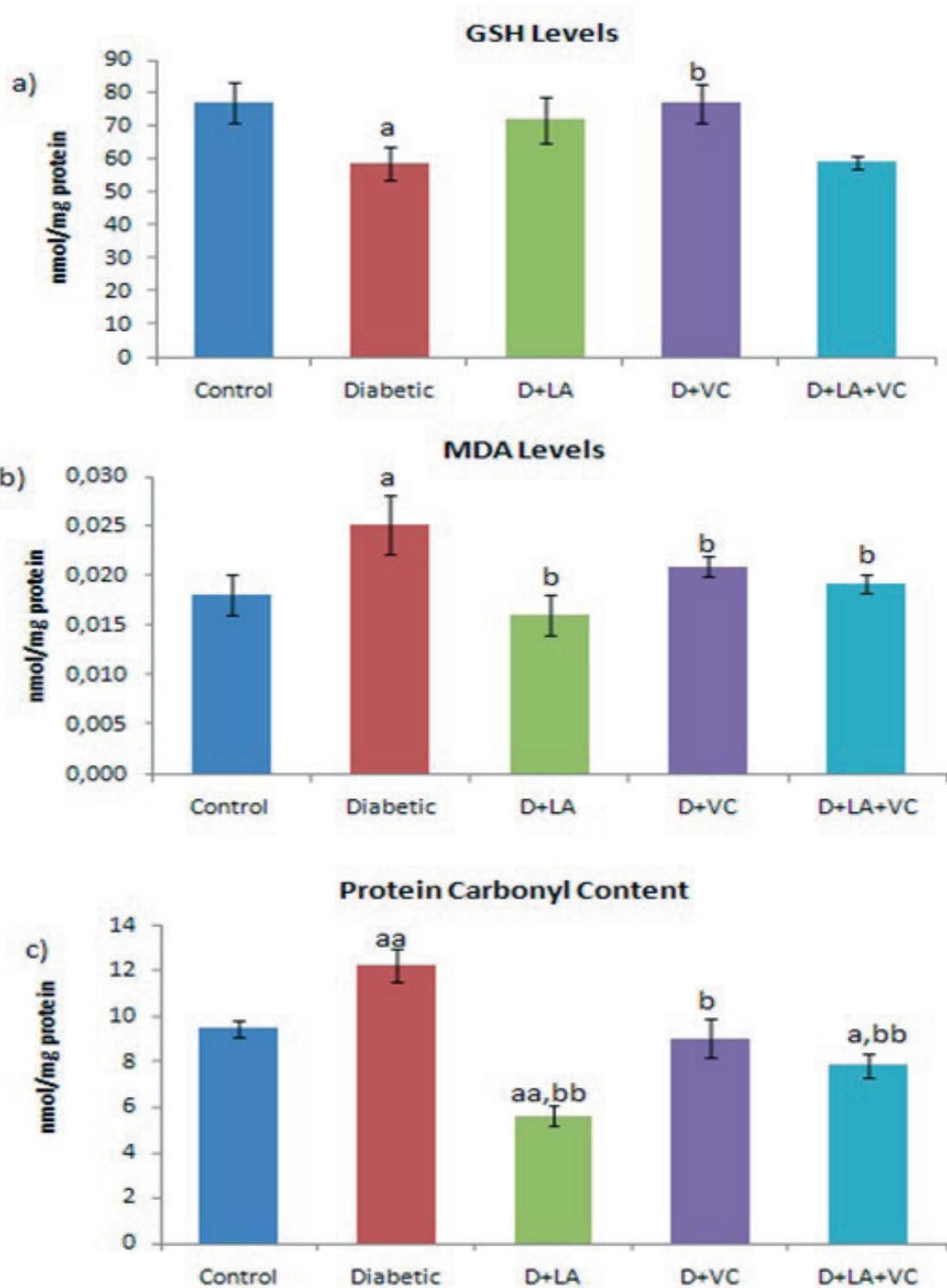
Figure 1: Liver tissue (a) GSH concentrations, (b) MDA contents and (c) protein carbonyl contents of control, diabetic, diabetic animals supplemented with α-lipoic acid (D+LA), diabetic animals supplemented with vitamin C (D+VC), and diabetic animals supplemented with both α-lipoic acid and vitamin C (D+LA+VC).

[a] represents significance at $p<0,05$ as compared with control groups.

[aa] represents significance at $p<0,005$ as compared with control groups.

[b] represents significance at $p<0,05$ as compared with untreated diabetic groups.

[bb] represents significance at $p<0,005$ as compared with untreated diabetic groups

Table 1. Summary of overall changes in tissue GSH levels, MDA (final product of lipid peroxidation) levels and protein carbonylation levels in control, diabetic, LA supplemented diabetic, VC supplemented diabetic and LA+VC supplemented diabetic rat liver tissues.

	N	GSH levels (nmol/mg protein)	MDA levels (nmol/mg protein)	Protien Carbonyls (nmol/mg protein)
Control	9	77,04±6,14	0,018±0,002	9,448±0,364
Diabetic	9	58,62±5,11 [a]	0,025±0,003 [a]	12,221±0,733 [aa]
Diabetic+ LA	8	71,85±6,90	0,016±0,002 [b]	5,595±0,452 [aa,bb]
Diabetic+ VC	12	76,95±5,96 [b]	0,021±0,001 [b]	9,018±0,876 [b]
Diabetic+ LA+VC	7	59,17±1,91	0,019±0,001 [b]	7,819±0,548 [a,bb]

Data were expressed as mean ± Standard Error of Mean (S.E.M)
[a]-represents significance at p<0,05, [aa]-represents significance at p<0,005 as compared with control groups.
[b]-represents significance at p<0,05,[bb]-represents significance at p<0,005 as compared with untreated diabetic groups.

Figure 2: Multiplex RT-PCR products of GST-Mu and β-actin genes amplified by using cDNAs synthesized from 1 µg total RNAs isolated from corresponding tissues. 500bp β-actin 450bp GST-Mu bands are clearly observed in control, diabetic, LA treated diabetic (D+LA), VC treated diabetic (D+VC) and LA+VC treated diabetic (D+LA+VC) groups.
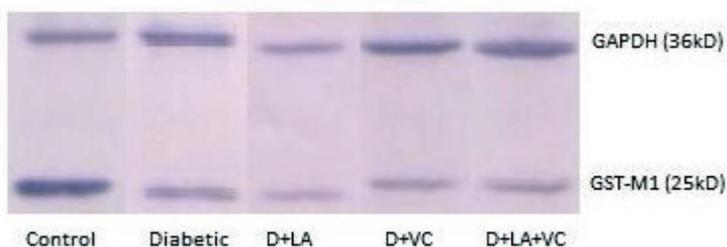
Figure 3: Western-blot analysis of GST-Mu and GAPDH proteins from corresponding tissues of individual rats belonging control, diabetic, LA treated diabetic (D+LA), VC treated diabetic (D+VC) and LA+VC treated diabetic (D+LA+VC) groups. On the membranes lower band represents 25kD GST-M1 protein and upper band represents 36kD GAPDH protein. 20 µg total proteins were loaded in each well and separated by 12% separating gel.
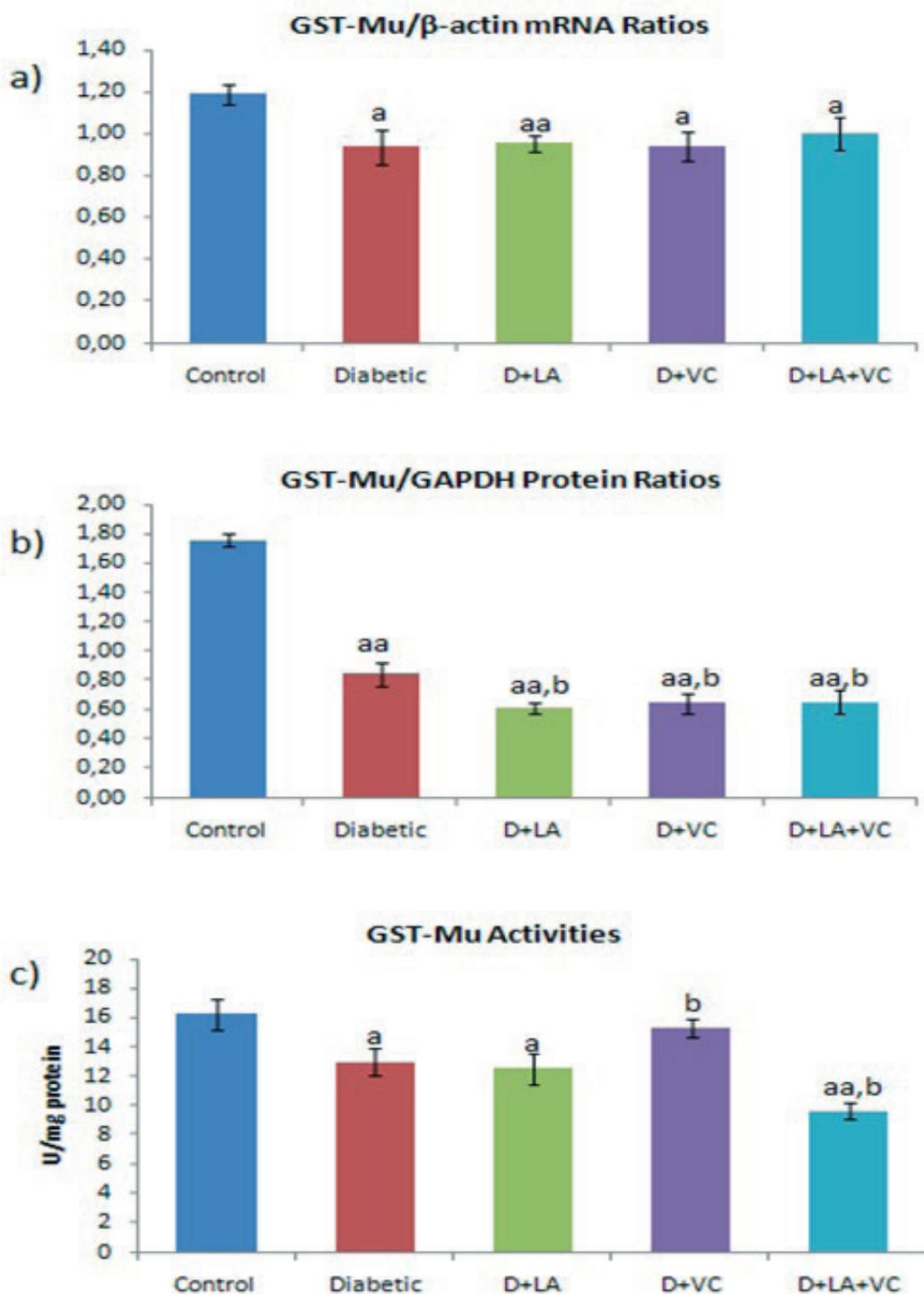
Figure 4: Bar diagrams of the results of GST-Mu (a) mRNA expressions, (b) protein expressions, and (c) the activities in control, diabetic, diabetic animals supplemented with α-lipoic acid (D+LA) and diabetic animals supplemented with vitamin C (D+VC), and diabetic animals supplemented with both α-lipoic acid and vitamin C (D+LA+VC).

[a] represents significance at $p<0,05$ as compared with control groups.
[aa] represents significance at $p<0,005$ as compared with control groups.
[b] represents significance at $p<0,05$ as compared with untreated diabetic groups.

Table 2. Summary of overall changes in the mRNA expressions, protein amounts and enzyme activities of GST-Mu isoform in control, diabetic and antioxidant supplemented diabetic rat liver tissues.

	N	GST-Mu mRNA (GST-Mu/β-actin)	GST-Mu Protein (GST-Mu/GAPDH)	GST-Mu Activity (U/mg protein)
Control	9	1,188±0,048	1,755±0,200	16,23±1,04
Diabetic	9	0,936±0,083 [a]	0,841±0,066 [aa]	12,96±0,89 [a]
Diabetic+ LA	8	0,953±0,037 [aa]	0,609±0,068 [aa,b]	12,49±1,04 [a]
Diabetic+ VC	12	0,941±0,069 [a]	0,645±0,044 [aa,b]	15,26±0,59 [b]
Diabetic+LA+VC	7	0,999±0,079 [a]	0,653±0,062 [aa,b]	9,59±0,56 [aa,b]

Data were expressed as mean ± Standard Error of Mean (S.E.M)
[a]-represents significance at $p<0,05$, [aa] represents significance at $p<0,005$ as compared with control groups.
[b]-represents significance at $p<0,05$

alone or in combination, did not change the mRNA expressions of GST-Mu in diabetic state.

For the determination of GST-Mu protein expression in tissues, Western-blot analysis was carried out by using rabbit anti GST-Mu IgG and the membranes were co-immunostained also by using rabbit anti GAPDH IgG. Figure 3 shows the results of multiplex Western-blot analysis of GST-Mu and GAPDH protein in control, diabetic, LA, VC and LA+VC treated diabetic rat liver tissues

The results showed that, there was a marked decrease in the relative protein expression of GST-Mu ($p<0,005$) in diabetic animals and neither antioxidants showed a restoring effect. Instead relative protein expressions were reduced further with antioxidant applications as shown in Figure 4b.

GST-Mu activities were measured in all groups by using DCNB as a specific substrate for that isoenzyme. Diabetes leads to a significant reduction in the activities of the enzyme ($p<0,05$) when compared to the control group. Although LA did not change GST-Mu activities, VC treatment caused a significant increase in the activities but still below the control values. Interestingly, when two antioxidants were given together, they reduced the GST-Mu activities further. Effects of diabetes and the antioxidant treatment on GST-Mu activities are shown in Figure 4c. Table 2 summarizes overall changes in the mRNA expressions, protein amounts and enzyme activities of GST-Mu isoenzyme in control, diabetic and antioxidant supplemented diabetic rat liver tissues.

Discussion

Diabetes mellitus is a group of metabolic diseases characterized by high blood glucose levels that could result from the defects in insulin secretion, or action, or both. Increased oxidative stress is playing an important role in the etiology and pathogenesis of the chronic complications associated with this disease. Numerous studies

proposed that, inhibition of oxidative stress with antioxidants might be an effective strategy for reducing the complications of diabetes. The beneficial effects of antioxidants in the treatment of diabetes have been shown by several investigators [19-23]. Although it is clear that oxidative stress plays a major role in diabetic complications, the molecular mechanisms of the effects of antioxidants especially on antioxidant and detoxification enzymes have not been clearly established. In the present study, we investigated the role of GST Mu expressions on the enzymatic activity and the role of strong antioxidants, LA and VC on the expressions and activity of the enzyme.

At first, we have showed the presence of oxidative stress during the diabetes in the liver tissues by measuring the extent of oxidative damage, namely by quantifying the oxidation products of the lipids and proteins, by measuring the MDA content and protein carbonylation levels respectively. Furthermore, the concentration of the major cellular antioxidant, reduced glutathione (GSH) was also quantified in diabetes and the effects of antioxidant treatments were evaluated in both control and diabetic rat liver tissues. Glucose oxidation, nonenzymatic glycation of proteins, and the subsequent oxidative degradation of glycated proteins observed in diabetics leads to inevitable productions of free radicals which in turn reduces the antioxidant defense mechanisms simultaneously leading to lipid peroxidation and hence damage the cells.

Reduced glutathione (GSH) is one of the most important biomolecules towards the toxicity induced by chemicals and free radicals and it is involved in the removal of reactive intermediates in the presence of GPx and GSTs with hidroperoxidation reactions. GSH has also free radical scavenging activity and repair the radical induced biological injuries. Previous studies have shown that, hepatic GSH concentration of STZ induced diabetic rats was significantly lower when compared with the normal rats [20,21,24]. According to our results, diabetes redu-

ced the GSH concentrations below the levels observed in control groups. Even though, individual LA and VC application brought the diabetic GSH state toward the control levels, their combined application yielded no significant changes in GSH concentration. The decrease in GSH in liver during diabetes most probably is the result of its increased utilization due to oxidative stress and the addition of antioxidants (LA or VC) slow down the excess usage of GSH. Since, LA has the ability to correct deficient thiol status of cells by increasing de novo synthesis of GSH and hence increased the GSH levels in diabetic animals [25].

Oxidative damage to unsaturated lipid molecules in a cell is a free radical induced phenomena leading to a process called lipid peroxidation. Even though low levels of lipid peroxides are present in a healthy cell, as the free radical concentration increases or the antioxidant system fails chain oxidation reactions of lipids results in enhanced lipid peroxidation. So, it is an important marker of early and reversible tissue damage caused by diabetes mellitus and decreased antioxidant defense capacity of the tissue. Lipid peroxidation also cause protein damage and inactivation of membrane bound enzymes either through direct attack by free radicals or through chemical modification by its end products which are MDA and 4-hydroxynonenal (4-HNE) [26]. According to our results, lipid peroxidation levels in the liver tissues of diabetic rats were significantly higher compared to controls and these findings are in agreement with the results of other related studies [23,27,28]. This marked increase in the lipid peroxidation rates in diabetic tissues suggests an accumulation of oxygen free radicals which can be due to either increased production and/or decreased elimination. Furthermore, application of antioxidants either alone or in combination reduced the levels towards the control values, most probably by decreasing the cellular redox potential.

The oxidative modification of proteins has been shown to play an important role in a number of human diseases [26]. Protein carbonyl content is actually a general indicator and the most commonly used marker of protein oxidation [29,30]. Accumulation of protein carbonyls has been observed in Alzheimer's disease, diabetes, inflammatory bowel disease, and arthritis [27,30]. Our results showed that, the carbonyl content of proteins in diabetic groups were higher compared to control groups, most probably, due to exposure of proteins to reactive oxygen species which could lead to modifications in amino acid side chains resulting in altered structure and/or functions. Other studies also showed that, the increase in the number of carbonyl groups within a protein correlated well with diabetes mellitus [27,31]. Application of LA and VC, alone or in combination decreased the level toward the control values. Effect of LA on the protein carbonylation was more prominent then the effect of VC alone. It reduced the protein carbonylation even below the control values.

Conjugating the glutathione to a wide range of electrophiles, Glutathione S-transferases also reveal diverse response to lots of chemicals and oxidative stress in the normal and disease conditions as diabetes mellitus [32]. Our data also demonstrated that, excessive oxidative stress occurred in the liver due to diabetes mellitus, resulted in the down regulation of the expression GST-Mu gene. We have previously reported that, the other main antioxidant enzymes such as SOD and CAT gene expressions were also suppressed in diabetes [12]. The decrease in the mRNA expressions of GST-Mu also reflected in both the protein expressions and the activities, indicating the transcriptional regulation of the gene. The reduction in the mRNA of GST-Mu isoenzyme in diabetes could be due to oxidation of transcriptional factors (e.g. Nrf2) responsible for the initiation machinery of antioxidant enzyme transcription process found in antioxidant response elements. The decrease could also be due to the decrease in the half lives of mRNAs since increased oxidative stress may lead to destabilization of mRNA [33]. Supplementing the diabetic animals with both LA and VC did not restore the gene and protein expressions of GST Mu. Furthermore, the level of protein synthesis were decreased drastically well below the diabetic values. But, especially VC treatment by itself increased GST Mu activities almost to control values through post translational mechanisms. Combinational effect of the two antioxidants was not so powerful even though it is known that LA also enhances the antioxidant properties of other antioxidants like VC in both aqueous and in hydrophobic environments [12].

In conclusion, we have found that GST Mu could be a marker enzyme in order to examine the effect of oxidative stress caused by diabetes. The results showed that oxidative stress dependent reduced cellular concentrations of glutathione, and increased oxidation-modified protein and lipid contents down regulated the gene and protein expressions of GST Mu. Antioxidants can restore the enzyme activities partially not through transcriptional regulation but by post translational mechanisms.

Acknowledgements

Ethical Issues

All experiments were carried out with the approval of local animal use committee (Fırat University, 02.08.2008 with 12/56 number). The procedures involving animals and their care are conformed to the institutional guidelines.

Conflict of Interest

The authors declare that they do not have any conflict of interest.

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