Interdiscip Toxicol. 2017; **Vol. 10**(4): 148–154.
doi: 10.1515/intox-2017-0021

ORIGINAL ARTICLE

Antioxidant SMe1EC2 modulates pentose phosphate pathway and glutathione-dependent enzyme activities in tissues of aged diabetic rats

Nuray Nuriye ULUSU [1], Müslüm GÖK [2], Arzu Ayşe SAYIN ŞAKUL [3], Nuray ARI [4], Milan STEFEK [5], Çimen KARASU [6], "The ADIC (Antioxidants in Diabetes-Induced Complications) Study Group"

[1] *Department of Medical Biochemistry, School of Medicine, Koc University, Istanbul, Turkey*
[2] *Department of Biochemistry, Faculty of Medicine, Hacettepe University, Ankara, Turkey*
[3] *Department of Pharmacology, Faculty of Medicine, Medipol University, Istanbul, Turkey*
[4] *Department of Pharmacology, Faculty of Pharmacy, Ankara University, Ankara, Turkey*
[5] *Department of experimental Pharmacology, Slovak Academy of Sciences, Bratislava, Slovakia*
[6] *Cellular Stress Response & Signal Transduction Research Laboratory, Department of Medical Pharmacology, Faculty of Medicine, Gazi University, Ankara, Turkey*

ITX100417A03 • Received: 27 November 2017 • Accepted: 10 December 2017

ABSTRACT

The pentose phosphate pathway and glutathione-associated metabolism are the main antioxidant cellular defense systems. This study investigated the effects of the powerful antioxidant SMe1EC2 (2-ethoxycarbonyl-8-methoxy-2,3,4,4a,5,9b-hexahydro-1H-pyrido[4,3-b] indolinium dichloride) on pentose phosphate pathway (PPP) and glutathione-dependent enzyme activities in aged diabetic and aged matched control rats. Diabetes was induced by streptozotocin injection in rats aged 13–15 months. Diabetic and control rats were divided into two subgroups, one untreated and one treated with SMe1EC2 (10 mg/kg/day, orally) for 4 months. SMe1EC2 ameliorated body weight loss, but not hyperglycemia of aged diabetic rats. Diabetes resulted in decreased glucose-6-phosphate dehydrogenase (G6PD), 6-phosphogluconate dehydrogenase (6PGD) and glutathione-S-transferase (GST), yet in unchanged glutathione reductase (GR) in the liver of aged diabetic rats. In the liver of the aged control rats, SMe1EC2 did not affect G6PDH, 6PGDH and GR, but it inhibited GST. SMe1EC2 also failed to affect diabetes-induced decline in 6PGDH, it ameliorated G6PDH but produced further decline in GST in the liver of aged diabetic rats. In the kidney of aged rats, G6PDH and GST were found to be comparable among the groups, but diabetes up-regulated 6PGDH and GR; these alterations were prevented by SMe1EC2. In the heart of aged diabetic rats, while GST remained unchanged, the recorded increase in G6PD, 6PGD, GR was prevented by SMe1EC2. Furthermore, an unchanged GR and remarkable increases in G6PD, 6PGD and GST were found in the lung of the aged diabetic group. These alterations were completely prevented by SMe1EC2. The results suggest that in aged rats SMe1EC2 can ameliorate the response of the kidney, heart and lung but not that of the liver against diabetes-induced glucotoxicity by interfering with the activity of redox network enzymes.

KEY WORDS: SMe1EC2; antioxidant; diabetes; aging; rat; pentose phosphate pathway; glutathione-dependent enzymes

ABBREVIATIONS:

SMe1EC2: 2-ethoxycarbonyl-8-methoxy-2,3,4,4a,5,9b-hexahydro-1H-pyrido[4,3-b]indolinium dichloride; **G6PD:** glucose-6-phosphate dehydrogenase; **6PGD:** 6-phosphogluconate dehydrogenase; **GST:** glutathione-S-transferase; **GR:** glutathione reductase; **GSSG:** oxidized glutathione; **ROS:** reactive oxygen species; **PPP:** pentose phosphate pathway; **NADPH:** reduced nicotinamide adenine dinucleotide phosphate; **Nrf2:** the nuclear factor erythroid 2-related factor 2; **STZ:** streptozotocin; **NOX:** NADPH oxidase

Correspondence address:
Prof. Çimen Karasu
Cellular Stress Response & Signal Transduction Research Laboratory
Faculty of Medicine, Gazi University, Turkey
TEL.: +90 312 2026921 • FAX +90 312 2124647
E-MAIL: cimenkrs@gmail.com, karasu@gazi.edu.tr

Introduction

Increases in the intracellular levels of reactive oxygen species (ROS), frequently referred to as oxidative stress, represent a potentially toxic insult. If not counteracted, it will attack lipids, sugars and proteins and oxidize them. Chronic oxidative stress, resulting in the accumulation of dysfunctional and damaged biomolecules, has numerous pathological consequences, including diabetes (Karasu, 2010; Stefek & Karasu, 2011; Şakul *et al.*, 2013). On the other hand, aging is caused by the accumulation of random molecular damage due to ROS (Ergin *et al.*, 2013; Cencioni *et al.*, 2013). The degenerative complications and deficits in organ performance as a result of enhanced susceptibility to the long-term effects of increased

oxidative stress and inflammation are increased by aging and diabetes. A decrease in endogenous antioxidant mechanisms with aging or diabetes increases the vulnerability of the tissues to oxidative damage (Shakeel, 2015; Panigrahy *et al.*, 2017). Because of the potential impact of oxidative stress and inflammatory vulnerability in aging and diabetes, research has focused on the use of synthetic anti-oxidant and anti-inflammatory agents that protect against stress-related phenomena (Karasu 2010, Aldini *et al.*, 2013). Many synthetic compounds have been evaluated as inhibitors of ROS formation, but none have yet been approved for clinical use.

In extensive preclinical studies, dietary supplementation with a potent antioxidant 2-ethoxycarbonyl-8-methoxy-2,3,4,4a,5,9b-hexahydro-1H-pyrido[4,3-b] indolinium dichloride (SMe1EC2), which is a hexahydropyridoindole derivative, revealed significant anti-oxidant, anti-inflammatory, vasculoprotective, antidysrhythmic and neuroprotective effects (Drimal *et al.*, 2008; Stefek *et al.*, 2013; Broskova *et al.*, 2013; Gasparova *et al.*, 2014). However, the regulating role of SMe1EC2 on pentose phosphate pathway (PPP) and glutathione-dependent enzymatic activities is not yet known, while PPP is one of the major sources of reduction equivalents for the glutathione peroxidase (GPx)/glutathione reductase (GR) antioxidant system, which plays a key role in preventing oxidative stress (Stanton, 2012; Ulusu, 2015). The present investigation was undertaken to assess the effects of SMe1EC2 treatment on the activities of glucose-6-phosphate dehydrogenase (G6PD), 6-phosphogluconate dehydrogenase (6PGD), glutathione reductase (GR) and glutathione-S-transferase (GST) in the liver, kidney, heart and lung of aged STZ diabetic rats.

Materials and methods

Animals and Treatment

Thirteen- to fifteen-month-old adult male Wistar rats were obtained from the "Laboratory Animal Unit of Ankara University, Faculty of Pharmacy". They were caged in groups of four with free access to food and water and were maintained on a 12-h light–dark cycle (7:00–19:00 h), at a temperature-controlled colony room (23±1 °C). These conditions were maintained constant throughout the experiments. All experimental procedures were performed in accordance with the National Institute of Health Guide for the Care and Use of Laboratory Animals (NIH publication number 80–23 revised 1996). Our research protocol was approved by the Ethical Committee for Animal Experimentation of the Ankara University (No: 2010-56-280).

Diabetes was induced in 13–15-month-old rats by two intravenous injections within an interval of two days of 2 × 20 mg/kg, i.p mg/kg Streptozotocin (STZ) in a 0.05 mol/l citrate buffer solution (Zúrová-Nedelcevová *et al.*, 2006). Ten days after treatment with STZ, tail vein blood glucose samples were measured with (Accu-check go®) to ensure induction of diabetes. The animals with a blood glucose level ≥250 mg/dl were accepted to be diabetic. Rats were given either SMe1EC2 (2-ethoxycarbonyl-8-methoxy-2,3,4,4a,5,9b-hexahydro-1H-pyrido[4,3-b]indolinium dichloride) (10 mg/kg/day) or vehicle (0.15 M saline) daily for 4 months by oral gavage (Sakul *et al.*, 2013). An initial 10-week period without treatment was introduced to avoid β-cell regeneration and alleviation of hyperglycemia, which is known to occur when antioxidants are administered together with streptozotocin or shortly after induction of diabetes (Koçak *et al.*, 2000).

Tissue homogenates

The tissues derived from the liver, kidney, heart and lung were kept in deep freeze (–85 °C) until the experiment date. Samples were homogenized in 10 mM Tris/HCl buffer (pH 7.6) containing 1 mM 2-Merkaptoetanol by using Ultraturrax IKA T18 homogenization (22 000 rpm/minute). After homogenization, samples were centrifuged in Eppendorf centrifuge 5417 R device, (20 800 rcf/25 min/4 °C). All of the steps mentioned above were completed on ice in order not to diminish the catalytic activity of the enzymes.

Glucose-6-phosphate dehydrogenase activity (G6PD)

All enzyme activities were determined spectrophotometrically using an ultraspec 2100 pro spectrophotometer at 340 nm and at 37 °C. G6PD was determined by monitoring the NADPH production at 340 nm and at 37 °C. The 500 µl assay mixture contained 50 µl of 2 mM NADP+ and 50 µl of 6 mM glucose-6-phosphate, 100 µl of 100 mM Tris/HCl buffer, pH 8.0 (containing 10 mM $MgCl_2$), 280 µl distilled water and 20 µl supernatant. Assays were carried out in duplicate and the activities were followed for 40 s. The reaction was linear during this period. One unit (U) of activity is the amount of enzyme required to reduce one µmol of NADP+ per min under the assay conditions. Specific activity was defined as units per mg of protein (Betke *et al.*, 1967).

6-phosphategluconate dehydrogenase activity (6PGDH)

6PGDH was measured by substituting 50 µl 0.6 mM 6-phosphogluconate as substrate in the assay mixture given above for G6PD measurement. The activity mixture contained 0.2 mM NADP+, 6 mM D-6-phosphateglucone-lactone (6PGA) as substrate in a 100 mM Tris/HCl+$MgCl_2$ (10 mM) buffer, pH 8.0. The enzyme activities were followed for 60 seconds (Pearse & Rosemeyer, 1975).

Glutathione-S-transferase activity (GST)

The tissue GST was measured by a previously described method using 1-choloro-2-4 dinitrobenzene as substrate. GST was determined by using 1 mM of glutathione (GSH), 1 mM of 1-chloro-2, 4-dinitrobenzene, 0.1 M of potassium phosphate buffer, pH 6.5, and tissue supernatants. The linear increase in absorbance at 340 nm was monitored at 37 °C. The enzyme activities were followed for 60 seconds (Habig *et al.*, 1981).

Glutathione reductase activity (GR)

GR was performed in the cytosolic fraction of tissue homogenates by monitoring the oxidation of NADPH in the presence of oxidized glutathione according to the previous method (Acan & Tezcan, 1989). The incubation mixture contained 100 mM sodium phosphate buffer, pH 7.4; 1 mM GSSG; 0.2 mM NADPH. Decrease in the absorbance of NADPH at 340 nm was monitored spectrophotometrically at 37 °C. A unit of activity (U) was defined as the amount of enzyme that catalyzes the oxidation of 1 μmol of NADPH in 1 min under these conditions (Tandogan *et al.*, 2011).

Protein determination

Protein content of the tissues was determined spectrophotometrically according to Bradford Coomassie brilliant blue G-250 assay using BSA as standard (Bradford, 1976).

Chemicals

SMe1EC2 was synthesized at the Institute of Experimental Pharmacology and Toxicology, Slovak Academy of Sciences, and was available as hydrochlorides. Tris [Tris (hydroxymethyl) aminomethane], Glucose-6-Phosphate (G6P), NADP+, 6-phosphogluconate (6PGA) were obtained from Sigma-Aldrich Chemical Co., MO, USA. BSA was obtained from Amersco Chemical Co., USA. All other chemicals were obtained from Sigma, USA.

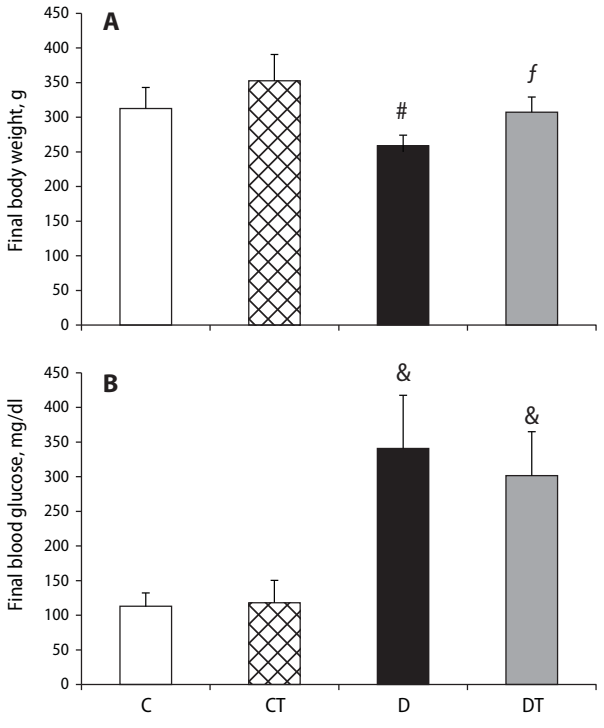

Figure 1. Effects of STZ-diabetes and SMe1EC2 treatment on final body weight (g; A) and final blood glucose (mg/dl; B) of rats. The rats were grouped as control (C), control treated (CT), diabetic (D), diabetic treated (DT). All data are expressed as the means ± SD. #$p<0.01$, &$p<0.001$ *vs.* control; ƒ$p<0.05$ *vs.* diabetic (ANOVA followed by Newman-Keuls test).

Statistical analysis

Data were expressed as mean ± SD. Statistical comparisons were carried out by one-way analysis of variance (ANOVA) and the Newman-Keuls test. A p-value <0.05 was considered statistically significant.

Results

Body weights and blood glucose levels of animals

The body weights and blood glucose levels of the animals before sacrifice are described in Figure 1. While diabetes resulted in loss of weight gain in aged rats, SMe1EC2 treatment produced a less but significant amelioration in the final body weight of aged diabetic rats. Diabetes also caused hyperglycemia that was not significantly affected by SMe1EC2, and the blood glucose levels of SMe1EC2-treated diabetic aged rats were found to be increased compared to aged control rats (Figure 1B).

Enzyme activities

Glucose-6-phosphate dehydrogenase activity (G6PD) significantly increased in the heart ($p<0.01$) and lung ($p<0.001$), was unchanged in the kidney ($p>0.05$), and decreased in the liver of aged diabetic rats compared to control rats treated or untreated with SMe1EC2. In the liver, heart and lung, SMe1EC2 treatment significantly prevented diabetes-induced alterations in G6PD in comparison with the aged diabetic untreated animals ($p<0.001$) (Figure 2A).

Diabetes resulted in a significant increase in kidney, heart and lung 6-phosphogluconate dehydrogenase activity (6PGD) ($p<0.001$) and a significant decrease in liver 6PGD ($p<0.001$). Treatment of aged diabetic animals with SME1EC2 significantly inhibited the increase of G6PD ($p<0.001$) but had no effect on liver G6PD in comparison with the aged diabetic untreated rats ($p>0.05$). SMe1EC2 treatment did not change liver, kidney, heart and lung G6PD in aged control rats ($p>0.05$) (Figure 2B).

Glutathione reductase activity (GR) did not change in the lung ($p>0.05$). It was elevated in the liver, kidney and heart of aged diabetic rats as compared to aged control rats ($p<0.001$). The treatment with SMe1EC2 led to a significant decrease in the liver, kidney and heart GR of diabetic rats compared to aged diabetic untreated rats ($p<0.001$). The treatment with SMe1EC2 did not significantly change GR in aged control animals (Figure 2C).

In the kidney and heart of aged rats, glutathione-S-transferase activity (GST) was not affected by diabetes ($p>0.05$). However, GST was significantly inhibited in the liver ($p<0.001$) and increased in the lung ($p<0.001$) of aged diabetic rats compared to aged control rats. SMe1EC2 treatment normalized lung GST ($p<0.001$) but not liver GST (Figure 2D). Liver GST was significantly decreased by SMe1EC2 treatment when compared to that of aged control and of aged diabetic group ($p<0.001$).

ISSN: 1337-6853 *(print version)* | 1337-9569 *(electronic version)*

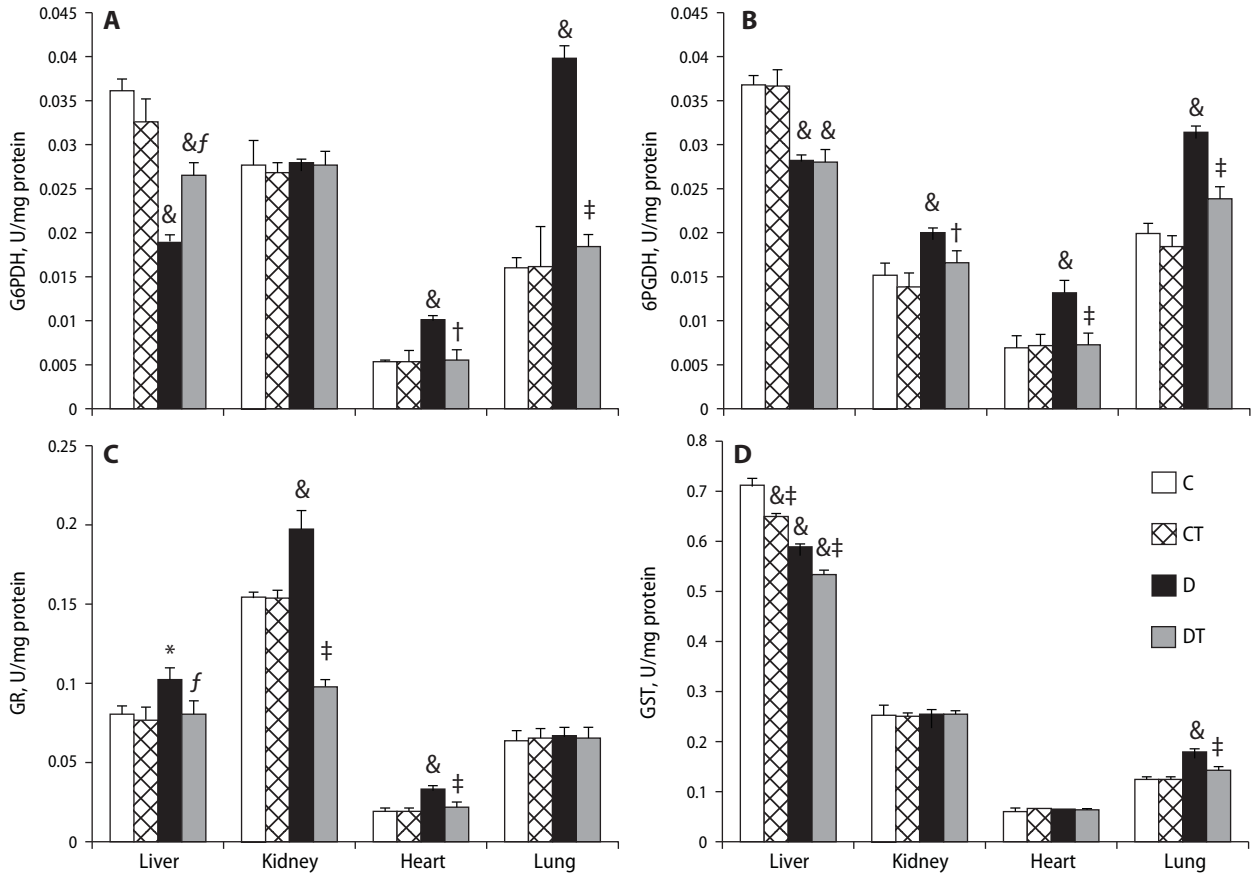

Figure 2. Effects of STZ-diabetes and SMe1EC2 treatment on glucose-6-phosphate dehydrogenase (G6PD), 6-phosphogluconate dehydrogenase (6PGD), glutathione reductase (GR) and glutathione-S-transferase activities in liver, kidney, heart and lung of rats. The rats were grouped as control (C), control treated (CT), diabetic (D), diabetic treated (DT). The enzyme activities were measured spectrophotometrically. All data are expressed as the means ± SD.*p<0.05, #p<0.01, &p<0.001 vs. control; ƒp<0.05, †p<0.01, ‡p<0.001 *vs.* diabetic (ANOVA followed by Newman-Keuls test).

Discussion

An accelerated formation of protein glyco-lipo-oxidation occurs in diabetes due to higher blood glucose. The long-term oxidation causes an inability of the proteins to do their function, which plays a key role in the pathogenesis of diabetic complications. Besides, oxidative stress is also the primary cause that links the loss of physiological functions with senescence. Thus the suppression of exacerbated oxidation is supposed to play an important role in the prevention and/or delay of diabetic complications and in the aging process. In this context, our group has undertaken a research program to assess the mechanisms of antioxidative, antiglycative and tissue protecting activity of synthetic pyridoindolic compounds for a long time. The present work is the first to investigate SMe1EC2 effects on PPP and glutathione-dependent enzyme activities, acting as the key modulators in the control of ROS, in aged diabetic rats.

We have already demonstrated that G6PD is increased in the brain, lung, pancreas and eye-lens of diabetic animals (Ulusu *et al.*, 2003; Ulusu *et al.*, 2005; Gök *et al.*, 2016). Accordingly, the present study showed an increased activation of heart and lung G6PD even in the presence of aging and diabetes. PPP generates NADPH and ribonucleotides, with enzymatic regulation by glucose-6-phosphate dehydrogenase (G6PDH), 6-phosphogluconolactonase (6PGL), and 6-phosphogluconate dehydrogenase (6PGDH). In fact, PPP is a main source for NADPH, which can be used as a cofactor of GR for the regeneration of GSH or as a substrate for NADPH oxidase for the release of free radicals. NADPH oxidases generally act as pro-oxidant enzymes, playing a crucial role in the pathogenesis of diabetes complications and the aging process. In our study, diabetes in aged rats led to a decrease in G6PDH, 6PGDH and GST only in the liver and no other tissues studied that displayed increased or unchanged G6PDH, 6PGDH or GST, suggesting the cell type-specific roles of PPP in the regulation of ROS balance, as indicated before (Park *et al.*, 2017). The decline in liver G6PDH, 6PGDH and GST may be due to excessive oxidative stress as indicated previously, showing that different types of

tissue increase the utilization of G6PDH and 6PGDH under oxidative stress conditions to allow efficient regeneration of NADPH and the reduction of glutathione disulfide (GSSG) to GSH via GR, which enhances the antioxidant defense system (Bolaños and Almeida, 2010). In accordance with this, we showed an up-regulated GR in the aged diabetic liver. Since G6PD is a key regulator responsible for intracellular redox homeostasis, in long-term conditions G6PD deficiency can alter redox balance leading to many abnormal cellular effects, such as the cellular inflammatory and immune response against viral infection (Yang *et al.*, 2016). Decreased G6PD activity and, as a result, decreased NADPH level may associate with liver disease in aged diabetics (Gupte *et al.*, 2009) since NADPH is required not only for the antioxidant system and NADPH oxidase but also for many essential cellular processes such as nitric oxide synthase and cytochrome p450 enzymes (Sahoo *et al.*, 2016).

Conversely, our study showed that G6PDH and 6PGDH were up-regulated along with increased or unchanged GR in the heart and lung of aged diabetic rats. This finding confirms our previous reports (Gök *et al.*, 2016) and is consistent with reports of others showing that high glucose concentrations leads to overexpression of G6PD and augmented PPP activity in different cell types (Peiró *et al.*, 2012). The overactivation of PPP may be linked with increased depletion of NADPH in these tissues due to chronic hyperglycemia via increased polyol pathway activity and upregulated aldose reductase (Kyselova *et al.*, 2005). However, the long-term overactivation of PPP would favor the utilization of NADPH by NADPH oxidase and the excess of free radical generation would further contribute to exhausting GSH (Peiró *et al.*, 2016). NADPH oxidase requires higher concentrations of NADPH to be active, as the Michaelis constant for this enzyme is five times higher than for glutathione reductase (Matsui *et al.*, 2005). It has been known for many years that diabetic patients have a decreased tissue concentration of GSH and the defects in GSH-dependent antioxidant enzymatic activity have been related to diabetes-associated tissue complications (Özdemir *et al.*, 2009; Raza *et al.*, 2012). In this context, we recall the early reports indicating that some tissues are highly sensitive even to low levels of hyperglycemia and NADPH than other tissues (Chandrasena *et al.*, 2008; Zhang *et al.*, 2010). It may be reasonable to explain the differences in enzymatic activities as the tissue's responses to aged diabetes.

It has been demonstrated that exacerbated tissue dysfunctions observed in diabetes were prevented both by NADPH oxidase inhibition or PPP blockade (Peiró *et al.*, 2016; Gök *et al.,* 2016). This is the first study showing that alterations in G6PD or 6PGDH due to aging and diabetes are prevented by a pyridoindol compound SMe1EC2 in the liver, kidney, heart and lung of rats. This is also consistent with our previous findings which showed that diabetes-induced regulations on PPP and glutathione-dependent enzymes were prevented by vitamin E and the antioxidant stobadine (Ulusu *et al.*, 2003).

In fact, there is but limited report indicating effects of aging on PPP and GSH-dependent enzymes. This stresses the importance of our work as the first study to evaluate PPP enzyme activities in different tissues of aged diabetic animals. Only one recent study reported that aging affected PPP and produced an age-dependent decrease in erythrocytes G6PDH, which was correlated with decreased GSH and increased oxidative stress (Maurya *et al.*, 2016). On the other hand, G6PD-Tg mice were shown to exhibit higher levels of NADPH, lower levels of ROS-derived damage, and better protection from aging-associated functional decline, including extended median lifespan in females. Nevertheless, the authors conclude that a modest increase in G6PD activity is beneficial for healthspan through increased NADPH levels and protection from the deleterious effects of ROS (Nóbrega-Pereira *et al.*, 2016).

By using a proteomic approach, it has been found that the regulatory role of G6PD in xenobiotic metabolism occurs possibly via NOX and the redox-sensitive Nrf2-signaling pathway to modulate the expression of xenobiotic-metabolizing enzymes (Yang *et al.*, 2016). In addition, GST, an important enzyme in detoxification, catalyzes the reaction of glutathione conjugation with many electrophilic xenobiotics (*e.g.* drugs, toxins, environmental pollutants, products of oxidative stress, and carcinogens) and their reactive metabolites formed via the cytochrome P-450 monooxygenase system. This enzyme was reported to be induced as a protective mechanism under conditions of increased oxidative stress, including diabetes (Pahwa *et al.*, 2017) and aging (Vyskočilová *et al.*, 2013) and favorable effects were achieved by treatment with antioxidants (Gök *et al.*, 2016; Chandran *et al.*, 2016). GST increased only in the lung, was unchanged in the kidney and heart and attenuated in the liver of aged diabetic rats. Except the liver, kidney and heart, only the lung showed an unaffected GR by diabetes and aging, while G6PDH and 6PGDH were up-regulated in the lung of aged diabetic rats. GST helps in lowering oxidative stress, thus the increment of GST in aged diabetic rats might be due to its utilization to alleviate the oxidative stress in the lung of aged diabetic rats. SMe1EC2 treatment also reduced GR in the kidney of aged diabetic rats below its normal level, implicating SMe1EC2-mediated dysregulation of reduction of the oxidized glutathione (GSSG).

GST activity depends on a steady quantity of GSH. If GSH is oxidized, it is reduced back by GR, which requires NADPH. Another interesting finding are the effects of SMe1EC2 treatment on liver GST. SMe1EC2 treatment lowered GST in the liver of aged rats in the presence or absence of diabetes, while it restored G6PDH, the rate-limiting enzyme of the pentose phosphate pathway, and GR to its normal levels in aged diabetic rats. With increasing age, a decline of biotransformation capacity was observed in laboratory animals and in man (Warrington *et al.*, 2004) and a marked fall in cytochrome p450 (CYP) activities. Oxidative biotransformation has been repeatedly described in old animals and humans (Yun *et al.*, 2010). Thus SMe1EC2-mediated increase in age- and

ISSN: 1337-6853 *(print version)* | 1337-9569 *(electronic version)*

diabetes-related decline in liver GST may have important clinical consequences by affecting both drug efficacy and toxicity. Nevertheless, we have already demonstrated that SMe1EC2 decreases age- and diabetes-induced up-regulation of oxidative protein modification in rat brain and peripheral tissues (Şakul *et al*., 2013).

Acknowledgements

This work was supported by VEGA (2/0041/15), SAS – TÜBİTAK Joint Project (No. JRP 2015/7), Ankara University BAP (10B336002) and Gazi University BAP (01/2010-126). and Gazi University BAP (01/2010-126).

Declaration of interest:
The authors report no conflict of interest. The authors alone are responsible for the content and writing of this paper.

REFERENCES

Acan NL, Tezcan EF. (1989). Sheep brain glutathione reductase: Purification and general properties. *FEBS Lett* **250**: 72–74.

Aldini G, Vistoli G, Stefek M, Chondrogianni N, Grune T, Sereikaite J, Sadowska-Bartosz I, Bartosz G. (2013). Molecular strategies to prevent, inhibit, and degrade advanced glycoxidation and advanced lipoxidation end products. *Free Radic Res* **47** Suppl 1: 93–137.

Betke K, Brewer GJ, Kirkman HN, Luzzato L, Motulsky AG, Ramot B, Siniscalco M. (1967). Standardized method for G-6-PD assay of haemolysates. *WHO Tech Rep Ser* **366**: 30–32.

Bolaños JP, Almeida A. (2010). The pentose-phosphate pathway in neuronal survival against nitrosative stress. *IUBMB Life* **62**: 14–18.

Bradford MM. (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein dye binding. *Anal Biochem* **72**: 248–254.

Broskova Z, Sotnikova R, Nedelcevova J, Bagi Z. (2013). Effect of a novel stobadine derivative on isolated rat arteries. *Interdiscip Toxicol* **6**: 63–66.

Cencioni C, Spallotta F, Martelli F, Valente S, Mai A, Zeiher AM, Gaetano C. (2013). Oxidative stress and epigenetic regulation in ageing and age-related diseases. *Int J Mol Sci* **14**: 17643–17663.

Chandran R, Parimelazhagan T, Shanmugam S, Thankarajan S. (2016). Antidiabetic activity of Syzygium calophyllifolium in Streptozotocin-Nicotinamide induced Type-2 diabetic rats. *Biomed Pharmacother* **82**: 547–554.

Chandrasena LG, De Silva LD, De Silva KI, Dissanayaka P, Peiris H. (2008). Changes in erythrocyte glucose-6-phosphate dehydrogenase (G6PD) and reduced glutathione (GSH) activities in the development of senile and diabetic cataracts. *Southeast Asian J Trop Med Public Health* **39**: 731–736.

Drimal J, Knezl V, Navarova J, Nedelcevova J, Paulovicova E, Sotnikova R, Snirc V, Drimal D. (2008). Role of inflammatory cytokines and chemoattractants in the rat model of streptozotocin-induced diabetic heart failure. *Endocr Regul* **42**: 129–135.

Gasparova Z, Stara V, Janega P, Navarova J, Sedlackova N, Mach M, Ujhazy E. (2014). Pyridoindole antioxidant-induced preservation of rat hippocampal pyramidal cell number linked with reduction of oxidative stress yet without influence on cognitive deterioration in Alzheimer-like neurodegeneration. *Neuro Endocrinol Lett* **35**: 454–462.

Gök M, Ulusu NN, Tarhan N, Tufan C, Ozansoy G, Arı N, Karasu Ç. (2016). Flaxseed Protects Against Diabetes-Induced Glucotoxicity by Modulating Pentose Phosphate Pathway and Glutathione-Dependent Enzyme Activities in Rats. *J Diet Suppl* **13**: 339–351.

Gupte RS, Floyd BC, Kozicky M, George S, Ungvari ZI, Neito V, Wolin MS, Gupte SA. (2009). Synergistic activation of glucose-6-phosphate dehydrogenase and NAD(P)H oxidase by Src kinase elevates superoxide in type 2 diabetic, Zucker fa/fa, rat liver. *Free Radic Biol Med* **47**: 219–228.

Habig WH, Jakoby WB. (1981). Glutathione transferase (human placenta). *Methods Enzymol* **77**: 218–231.

Karasu C. (2010). Glycoxidative stress and cardiovascular complications in experimentally-induced diabetes: effects of antioxidant treatment. *Open Cardiovasc Med J* **4**: 240–256.

Koçak G, Aktan F, Canbolat O, Ozoğul C, Elbeğ S, Yildizoglu-Ari N, Karasu C; ADIC Study Group-Antioxidants in Diabetes-Induced Complications. (2000). Alpha-lipoic acid treatment ameliorates metabolic parameters, blood pressure, vascular reactivity and morphology of vessels already damaged by streptozotocin-diabetes. *Diabetes Nutr Metab* **13**: 308–318.

Kyselova Z, Gajdosik A, Gajdosikova A, Ulicna O, Mihalova D, Karasu C, Stefek M. (2005). Effect of the pyridoindole antioxidant stobadine on development of experimental diabetic cataract and on lens protein oxidation in rats: comparison with vitamin E and BHT. *Mol Vis* **11**: 56–65.

Matsui R, Xu S, Maitland KA, Hayes A, Leopold JA, Handy DE, Loscalzo J, Cohen RA. (2005). Glucose-6 phosphate dehydrogenase deficiency decreases the vascular response to angiotensin II. *Circulation* **112**: 257–263.

Maurya PK, Kumar P, Chandra P. (2016). Age-dependent detection of erythrocytes glucose-6-phosphate dehydrogenase and its correlation with oxidative stress. *Arch Physiol Biochem* **122**: 61–66.

Nóbrega-Pereira S, Fernandez-Marcos PJ, Brioche T, Gomez-Cabrera MC, Salvador-Pascual A, Flores JM, Viña J, Serrano M. (2016). G6PD protects from oxidative damage and improves healthspan in mice. *Nat Commun* **7**: 10894.

Ozdemir S, Tandogan B, Ulusu NN, Turan B. (2009). Angiotensin II receptor blockage prevents diabetes-induced oxidative damage in rat heart. *Folia Biol (Praha)* **55**: 11–16.

Pahwa S, Sharma R, Singh B. (2017). Role of Glutathione S-Transferase in Coronary Artery Disease Patients with and Without Type 2 Diabetes Mellitus. *J Clin Diagn Res* **11**: BC05–BC08.

Panigrahy SK, Bhatt R, Kumar A. (2017). Reactive oxygen species: sources, consequences and targeted therapy in type 2 diabetes. *J Drug Target* **25**: 93–101.

Park YJ, Choe SS, Sohn JH, Kim JB. (2017). The role of glucose-6-phosphate dehydrogenase in adipose tissue inflammation in obesity. *Adipocyte* **8**: 1–7.

Pearse BMF, Rosemeyer MA. (1975). 6-Phosphogluconate dehydrogenase from human erythrocytes. *Methods Enzymol* **41**: 220–226.

Peiró C, Romacho T, Azcutia V, Villalobos L, Fernández E, Bolaños JP, Moncada S, Sánchez-Ferrer CF. (2016). Inflammation, glucose, and vascular cell damage: the role of the pentose phosphate pathway. *Cardiovasc Diabetol* **15**: 82.

Raza H, John A, Howarth FC. (2012). Alterations in glutathione redox metabolism, oxidative stress, and mitochondrial function in the left ventricle of elderly Zucker diabetic fatty rat heart. *Int J Mol Sci.* **13**: 16241–16254.

Sahoo S, Meijles DN, Pagano PJ. (2016). NADPH oxidases: key modulators in aging and age-related cardiovascular diseases? *Clin Sci* **130**: 317–335.

Sakul A, Cumaoğlu A, Aydin E, Ari N, Dilsiz N, Karasu C. (2013) Age- and diabetes-induced regulation of oxidative protein modification in rat brain and peripheral tissues: consequences of treatment with antioxidant pyridoindole. *Exp Gerontol* **48**: 476–484.

Shakeel M. (2015). Recent advances in understanding the role of oxidative stress in diabetic neuropathy. Diabetes *Metab Syndr* **9**: 373–378.

Stanton RC. (2012). Glucose-6-phosphate dehydrogenase, NADPH, and cell survival. *IUBMB Life* **64**: 362–369.

Stefek M, Karasu C. (2011). Eye lens in aging and diabetes: effect of quercetin. *Rejuvenation Res* **14**: 525–534.

Stefek M, Milackova I, Juskova-Karasova M, Snirc V. (2013). Antioxidant action of the hexahydropyridoindole SMe1EC2 in the cellular system of isolated red blood cells *in vitro*. *Redox Rep* **18**: 71–75.

Tandogan B, Güvenç A, Çalış İ, Ulusu NN. (2011). *In vitro* effects of compounds isolated from Sideritis brevibracteata on bovine kidney cortex glutathione reductase. *Acta Biochim Pol* **58**: 471–475.

Ulusu NN, Ozbey G, Tandogan B, Gunes A, Durakoglugil DB, Karasu C, Uluoglu C, Zengil H. (2005). Circadian variations in the activities of 6-phosphogluconate dehydrogenase and glucose-6-phosphate dehydrogenase in the liver of control and streptozotocin-induced diabetic rats. *Chronobiol Int* **22**: 667–677.

Ulusu NN, Sahilli M, Avci A, Canbolat O, Ozansoy G, Ari N, Bali M, Stefek M, Stolc S, Gajdosik A, Karasu C. (2003). Pentose phosphate pathway, glutathione-dependent enzymes and antioxidant defense during oxidative stress in diabetic rodent brain and peripheral organs: effects of stobadine and vitamin E. *Neurochem Res* **28**: 815–823.

Ulusu NN. (2015). Glucose-6-phosphate dehydrogenase deficiency and Alzheimer's disease: Partners in crime? The hypothesis. *Med Hypotheses* **85**: 219–223.

Vyskočilová E, Szotáková B, Skálová L, Bártíková H, Hlaváčová J, Boušová I. (2013). Age-related changes in hepatic activity and expression of detoxification enzymes in male rats. *Biomed Res Int* **2013**: 408573.

Warrington JS, Greenblatt DJ, von Moltke LL. (2004). Age-related differences in CYP3A expression and activity in the rat liver, intestine, and kidney. *J Pharmacol Exp Ther* **309**: 720–729.

Yang HC, Wu YH, Liu HY, Stern A, Chiu DT. (2016). What has passed is prolog: new cellular and physiological roles of G6PD. *Free Radic Res* **50**: 1047–1064.

Yun KU, Oh SJ, Oh JM, Kang KW, Myung CS, Song GY, Kim BH, Kim SK. (2010). Age-related changes in hepatic expression and activity of cytochrome P450 in male rats. *Arch Toxicol* **84**: 939–946.

Zhang Z, Liew CW, Handy DE, Zhang Y, Leopold JA, Hu J, Guo L, Kulkarni RN, Loscalzo J, Stanton RC. (2010). High glucose inhibits glucose-6-phosphate dehydrogenase, leading to increased oxidative stress and beta-cell apoptosis. FASEB J **24**: 1497–1505.

Zúrová-Nedelcevová J, Navarová J, Drábiková K, Jancinová V, Petríková M, Bernátová I, Kristová V, Snirc V, Nosál'ová V, Sotníková R. (2006). Participation of reactive oxygen species in diabetes-induced endothelial dysfunction. *Neuro Endocrinol Lett* **27** Suppl 2:168–171.

ISSN: 1337-6853 *(print version)* | 1337-9569 *(electronic version)*