Journal of Animal and Veterinary Advances 12 (9): 927-931, 2013
ISSN: 1680-5593

Oxidative Stress Induced by Diabetes Activates Apoptosis in Rat Brain

[1]Hasan Aksit, [2]Kamil Seyrek, [3]Funda Kiral, [3]Pinar Alkim Ulutas and [3]Turgut Sekerler
[1]Department of Biochemistry, Faculty of Veterinary Medicine,
[2]Department of Medical Biochemistry, Faculty of Medicine,
University of Balikesir, Balikesir, Turkey
[3]Department of Biochemistry, Faculty of Veterinary Medicine,
University of Adnan Menderes, Aydin, Turkey

Abstract: Diabetes is an increasing worldwide health problem. It is well known that diabetes causes brain damage. The mechanism by which diabetes increases brain damage is still elusive. The objective of this study was to determine the effects of streptozotocin-induced diabetes on rat brain tissue. Twenty rats were divided into control and experimental groups at random. In experimental animals diabetes was induced by intraperitoneal injection of a single 50 mg kg^{-1} dose of streptozotocin while the animals in control group received sodium citrat buffer. Induction of diabetes with streptozotocin resulted in a statistically significant increase in the glucose and malondialdehyde levels whereas ascorbic acid concentrations and Total Antioxidant Status (TAS) decreased significantly in these animals. Compared to the control animals hyperglycemia induced apoptosis in brain tissue of experimental rats significantly. In conclusion, in streptozotocin induced hyperglycaemia in diabetic rats leads to oxidative stress and induce apoptosis in brain tissue. In addition, increased rate of apoptosis might be due to elevated concentrations of reactive oxygen species.

Key words: Apoptosis, diabetes, MDA, rat, TAS

INTRODUCTION

Diabetes Mellitus (DM) is a group of common metabolic and degenerative diseases clinically characterized by a phenotype of increased blood glucose, decreased insulin secretion and/or resistance and impairments in carbohydrate, fat and protein metabolism, associated with a variety of pathophysiological complications (Rahimi *et al*., 2005; Bingol and Kocamis, 2010; Lumini-Oliveira *et al*., 2010). DM is a significant healthcare concern worldwide that affects >165 million individuals leading to cardiovascular disease, nephropathy, retinopathy and widespread disease of both the peripheral and central nervous systems (Maiese *et al*., 2007).

Apoptosis or physiological cell death is characteristic of diabetes and have been demonstrated in various tissues including the cardiovascular system, the retina and the central nervous system (Feldman *et al*., 1997). The presence of apoptotic neurons in diabetic animals has been correlated with serum glucose concentraions, suggesting hyperglycemia as a major factor that enhances apoptosis (Vincent and Maiese, 1999; Gurpinar *et al*., 2010). However, little is known about the mechanism by which hyperglycemia affects neurons (Sharifi *et al*., 2007). It has been reported that impairments in memory, learning and cognition are more common in diabetic people than in nondiabetic ones. Recent studies have proposed that neuronal apoptosis may also play a significant role in the pathogenesis of diabetic sensory and autonomic neuropathies (Reaven *et al*., 1990; Li *et al*., 2005).

Production of Reactive Oxygene Species (ROS) can lead to cell injury through a number of processes that involve the peroxidation of cellular membrane lipids (Siu and To, 2002), the peroxidation of docosahexaenoic acid, a precursor of neuroprotective docosanoids (Mukherjee *et al*., 2004) and the oxidation of proteins that yield protein carbonyl derivatives and nitrotyrosine (Adams *et al*., 2001). ROS have been implicated in the apoptosis of a variety of cell types that involve neurons, endothelial cells, cardiomyocytes and smooth muscle cells through multiple cellular pathways (Buttke and Sandstrom, 1994). Apoptotic cells exibit internucleosomal DNA cleavage and this occurred at sites between nucleosoms, protein containing structures that occur in chromatin at ~200 bp intervals (Hughes and Cidlowski, 1994).

Corresponding Author: Hasan Aksit, Department of Biochemistry, Faculty of Veterinary Medicine, University of Balikesir, Balikesir, Turkey

The brain is the tissue most vulnerable to oxidative damage because of its high rate of oxidative metabolic activity, intensive production of reactive oxygen metabolites, relatively low antioxidant capacity, low repair mechanism activity, nonreplicating nature of its neuronal cells and the high membrane surface to cytoplasm ratio (Rice-Evans and Burdon, 1993). Polyunsaturated fatty acids, highly abundant in the brain, serve as a major biological target for the oxidative damage induced by ROS. Lipid peroxidation is a well-established mechanism of cellular injury and is used as an indicator of oxidative stress in cells and tissues (Magni *et al*., 1994; Yenisey *et al*., 2006; Akbay *et al*., 2011). Measurement of Malodialdehyde (MDA) is widely used as an indicator of lipid peroxidation (Kawase *et al*., 1989).

Ascorbic acid, also known as vitamin C is involved in numerous biological processes including scavenging free radicals to prevent oxidative damage, acting as a cofactor for reactions such as hydroxylation of proline necessary for collagen needed for blood vessels and wound healing, hormone synthesis, iron absorption and immune system function, among others. It serves as a strong reducing agent by donating electron (s) thus directly neutralizing ROS and it also acts to recycle the tocopherol radical to its active, reduced form (Seyrek *et al*., 2010).

Detection of Total Antioxidant Status (TAS) is an easy and reliable method. All of the antioxidants including water and lipid soluble vitamins, proteins, lipids, gluthatione and uric acid are assesed together in this protocol (Guldiken *et al*., 2009). Despite of extensive investigations, the mechanism (s) activating apoptotic pathways in the diabetic brain have not been completely understood (Li *et al*., 2004). The aim of the present study was to investigate the effects of streptozotocin-induced diabetes on rat brain tissue.

MATERIALS AND METHODS

All studies on the animals described in the current study were reviewed and approved by University of Adnan Menderes Institutional Animal Ethic Committee (Date: 16.06.2009, No: B.30.2.ADU.0.06. 00.00/124-HEK/2009/025).

Animals: Twenty male Sprague-Dawley rats were used in this study. Rats were randomly divided into two groups, the control and diabetic groups, each containing ten animals. All animals were kept in standard rat cages, 5 rats per cage and housed in an air-conditioned room with controlled lighting (12 h light/dark). All rats were allowed free access to water and rat chow.

Induction of diabetes: At the beginning of the experiment blood glucose concentrations of all animals were determined using a glucometer (Medisense-Optium, UK). Diabetes was induced in overnight fasted experimental group by a single intraperitoneal injection of streptozotocin dissolved in citrate buffer (pH 4.5) at a dose of 50 mg kg^{-1} while the control group was injected with the citrate buffer alone. To confirm the presence of hyperglycemia at the end of 3 weeks, blood was collected from the tip of the tail under light ether anaesthesia and rats were killed by cervical dislocation. If blood glucose in the tail vein exceeded 250 mg dL^{-1} animals were considered hyperglycaemic.

Biochemical analyses: The measurement of cytoplasmic histone-associated DNA fragments after induction of cell death was performed with the Cell Detection ELISA plus (Manheim, Germany). Briefly, cytoplasmic lysates from brain of control and diabetic rats were transfered to a streptavidin coated plate supplied by the manufacturer. Plate was read for optical density at 405 nm.

The MDA production and hence lipid peroxidation was assessed in serum and tissue samples as described previously (Ohkawa *et al*., 1979). The end product of lipid peroxidation formed a coloured product with thiobarbituric acid measurable at 532 nm (Shimadzu, UV-1601).

Serum and tissue ascorbic acid was measured by the Phosphotungstic Acid Method of Kyaw (1978). For erythrocyte separation, heparinized blood was centrifuged at 3500 rpm for 10 min to separate the red blood cells from the plasma. Tissues samples were homogenised in Phosphat Buffer Saline (PBS) (pH 7.4) and centrifuged at 6000 g for 10 min. Supernatants were used for ascorbic acid detection.

TAS in brain tissue and serum was measured using commercial available kit (Rel Assay Diagnostics, Gaziantep/Turkey) with a microtiter plates and an ELISA reader (Anthos, 2010, Anthos Labtec Instruments, Salzburg, Austria). The analyses were carried out according to the manufacturer's instructions.

Statistics: The data was analysed using the Student's t-test (for Windows Release 12 Standard Version Copyright© SPSS Inc. 1989-2001) and $p<0.05$ was considered as statistically significant.

RESULTS

Brain and serum glucose concentrations, rate of apoptosis in brain tissue, brain and serum MDA levels as well as brain and serum TAS of control and diabetic rats are given in Table 1.

Table 1: Brain and serum glucose concentrations, rate of apoptosis in brain tissue, brain and serum MDA levels as well as brain and serum TAS of control and diabetic rats. Results are expressed as means±standard deviations ($\bar{X}\pm S_{\bar{x}}$)

	($\bar{X}\pm S_{\bar{x}}$)		
Parameters	Control group (n = 10)	Experimental group (n = 10)	p-value
Serum glucose (mg dL^{-1})	174.20±21.13	376.30±40.65	<0.050
Apoptosis rate in brain (Absorbance at 405 nm, U)	0.33±0.240	0.73±0.390	<0.050
Brain MDA (μmol g^{-1} protein)	2.97±1.060	3.86±2.830	>0.050
Serum MDA (μmol L^{-1})	16.21±2.850	19.30±3.460	<0.050
Brain ascorbic acid (mg g^{-1} protein)	5.60±1.850	4.07±0.840	<0.050
Serum ascorbic acid (mg L^{-1})	11.28±1.870	6.78±1.970	<0.001
Brain TAS (mmol trolox equivalent g^{-1} protein)	0.54±0.190	0.38±0.080	<0.050
Serum TAS (mmol trolox equivalent L^{-1})	3.25±0.420	2.85±0.280	<0.050

Intraperitonal administration of streptozotocin resulted in a statistically significant increase serum glucose concentration in diabetic rats ($p<0.05$). As shown in Table 1, the excess concentration of glucose induced DNA-fragmentation, a hallmark of apoptosis in brain cells of diabetic rats. Hyperglycemia caused also an increase in MDA levels, an indicator of lipid peroxidation both in brain tissue and in serum of diabetic rats. In terms of brain and serum ascorbic acid levels there was a significant decrease ($p<0.05$) in diseased animals. Regarding the brain and serum TAS a statistical significant ($p<0.05$) decrease was observed in diabetic rats.

DISCUSSION

During cellular injury, oxygen free radicals can be produced in significant quantities during the reduction of oxygen and ultimately result in oxidative stress with cell death. ROS consist of oxygen free radicals and other chemical entities that include superoxide free radicals, hydrogen peroxide, singlet oxygen, nitric oxide and peroxynitrite (Chong *et al*., 2005; Maiese *et al*., 2007). Most species are produced at low levels during normal physiological conditions and are scavenged by endogenous antioxidant systems that include superoxide dismutase, glutathione peroxidase, catalase and small molecule substances such as vitamins C and E (Chong *et al*., 2005). It is well known that diabetes stimulates free radical production and thus aggravates brain damage.

Diabetes is a metabolic disorder that has been shown to adversely affect both the central nervous systems and peripheral nervous system by increasing basal neuronal apoptosis (Britton *et al*., 2003; Li *et al*., 2005). A chronic degenerative change in the nervous system is one of the hallmarks of diabetes (Barber *et al*., 1998).

Regardless of the diabetes type, highly conserved intracellular pathways of apoptosis are triggered. These molecular mediators of β-cell apoptosis and the intracellular pathways activated. Endoplasmic Reticulum (ER) stress is increasingly acknowledged as an important mechanism in the development of diabetes. ER stress is important not only for β-cell loss but also for insulin resistance. They highlight the role of ER stress-induced apoptosis in liver and adipose tissue and discuss the exact interactions between environmental signals, ER stress and apoptosis in these organs (Krijnen *et al*., 2009).

In the present study, researchers demonstrated that streptozotocin induced hyperglycemia stimulates oxidative stress in brain tissue and serum of experimental rats. The observations are consistent with those of other investigators (Li *et al*., 2006) who demonstrated that activated ROS production in diabetes results in increased rate of apoptosis.

Ascorbic acid levels in serum were found to be significantly ($p<0.001$) reduced in diabetic animals. This finding is in line with the reports of earlier investigators (Maxwell *et al*., 1997; Samiec *et al*., 1998). The reason of these reduced ascorbic acid levels in experimental animals is unclear. This might be in part due to the using of ascorbic acid for elimination of increased ROS in hyperglycemic rats. It is a well known fact that ascorbic acid serves as a strong reducing agent by donating electron (s) thus directly neutralizing ROS and it also acts to recycle the tocopherol radical to its active, reduced form (Bildik *et al*., 2004). Similarly, tissue ascorbic acid concentrations in brain of diabetic rats were significantly ($p<0.05$) lower than that of controls. This result indicates that hyperglycemia reduces the supply of ascorbic acid to the brain. Thus, low levels of tissue ascorbic acid in brain of diabetic rats may render brain tissue of these animals particularly at risk for ROS-induced damage.

Once ascorbate has been depleted, the remaining antioxidants provide only partial protection from ROS which may interact with lipoproteins and initiate lipid peroxidation (Magni *et al*., 1994). The end product of lipid peroxidation is MDA which is measured conveniently (Biswas *et al*., 1995, 1997). Lipid peroxidation causes damage to biological membranes and nerves and leads to production of secondary reactive metabolites (e.g., aldehydes). Other biological targets may be proteins which can sustain direct damage or chain reaction analogues of fatty acids (Stadtman, 1993). As might be expected, researchers have found that the excess concentration of glucose induced serum lipid peroxidation in diabetic rats. It is somewaht surprising, however that a

statisticaly significant increase in MDA levels was observed in serum but not in brain tissue of diabetic rats. It is well known that cells in brain are particularly sensitive to ROS (Magni *et al*., 1994). Since, polyunsaturated fatty acids, highly abundant in the brain, serve as a major biological target for the oxidative damage induced by ROS (Rice-Evans and Burdon, 1993). The reason of this relatively moderate increase in MDA levels in brain tissue of diabetic rats remains to be elucidated.

CONCLUSION

Results of the present study showed that serum and brain TAS of diabetic rats was significantly lower than that of controls. Due to the low level of TAS, cells in tissues of diabetic rats could be at greater risk of oxidative damage than cells in non-diabetic ones (Valko *et al*., 2007). It is known that oxidative stress represents a significant mechanism for the destruction of cells that can involve apoptotic cell injury. Similar to this statement, recent clinical observations suggest that diabetes is correlated with the erosion of certain brain function such as impaired performance in global memory, attention, abstract reasoning and visual-motor tasks (Ryan and Geckle, 2000; Martinez-Tellez *et al*., 2005). Therefore, consideration of antioxidants in clinical treatment as adjunct therapy in diabetes could be recommended.

REFERENCES

Adams, S., P. Green, R. Claxton, S. Simcox, M.V. Williams, K. Walsh and C. Leeuwenburgh, 2001. Reactive carbonyl formation by oxidative and non-oxidative pathways. Front Biosci., 6: 17-24.

Akbay, T.T., A. Yarat, O. Sacan and R. Yanardag, 2011. The effect of glurenorm (gliquidone) on aorta in STZ induced diabetic rats. Kafkas Univ. Vet. Fak. Derg., 17: 235-238.

Barber, A.J., E. Lieth, S.A. Khin, D.A. Antonetti, A.G. Buchanan and T.W. Gardner, 1998. Neural apoptosis in the retina during experimental and human diabetes: Early onset and effect of insulin. J. Clin. Invest., 102: 783-791.

Bildik, A., F. Kargin, K. Seyrek, S. Pasa and S. Ozensoy, 2004. Oxidative stress and non-enzymatic antioxidative status in dogs with visceral leishmaniasis. Res. Vet. Sci., 77: 63-66.

Bingol, S.A. and H. Kocamis, 2010. Saglikli ve diabet oluþturulmuþ farelerin bobrek dokusunda katalaz enziminin RT-PCR ile gen ve immunohistokimyasal olarak protein ekspresyonu. Kafkas Univ. Vet. Fak. Derg., 16: 825-834.

Biswas, T., D.K. Ghosh, N. Mukherjee and J. Ghosal, 1997. Lipid peroxidation of erythrocytes in visceral Leishmaniasis. J. Parasitol., 83: 151-152.

Biswas, T., J.K. Pal, K. Naskar, D.K. Ghosh and J. Ghosal, 1995. Lipid peroxidation of erythrocytes during anemia of the Hamsters infected with *Leishmania donovani*. Mol. Cell Biochem., 146: 99-105.

Britton, M., J. Rafols, S. Alousi and J.C. Dunbar, 2003. The effects of middle cerebral artery occlusion on central nervous system apoptotic events in normal and diabetic rats. Int. J. Exp. Diabesity Res., 4: 13-20.

Buttke, T.M. and P.A. Sandstrom, 1994. Oxidative stress as a mediator of apoptosis. Immunol. Today, 15: 7-10.

Chong, Z.Z., F. Li and K. Maiese, 2005. Oxidative stress in the brain: Novel cellular targets that govern survival during neurodegenerative disease. Prog. Neurobiol., 75: 207-246.

Feldman, E.L., M.J. Stevens and D.A. Greene, 1997. Pathogenesis of diabetic neuropathy. Clin. Neurosci., 4: 365-370.

Guldiken, B., M. Demir, S. Guldiken, N. Turgut, B. Turgut and A. Tugrul, 2009. Oxidative stress and total antioxidant capacity in diabetic and nondiabetic acute ischemic stroke patients. Clin. Appl. Thromb. Hemost., 15: 695-700.

Gurpinar, T., N. Ekerbicer, N. Uysal, T. Barut, F. Tarakci and M.I. Tuglu, 2010. The histologic evaluation of atorvastatin and melatonin treatment on oxidative stress and apoptosis of diabetic rat pancreas. Kafkas Univ. Vet. Fak. Derg., 16: 547-552.

Hughes, F.M. and J.A. Cidlowski, 1994. Apoptotic DNA degradation: Evidence for novel enzymes. Cell Death Differ., 1: 11-17.

Kawase, T., S. Kato and C.S. Lieber, 1989. Lipid peroxidation and antioxidant defense systems in rat liver after chronic ethanol feeding. Hepatology, 10: 815-821.

Krijnen, P.A.J., S. Simsek and H.W.M. Niessen, 2009. Apoptosis in diabetes. Apoptosis, 14: 1387-1388.

Kyaw, A., 1978. A simple colorimetric method for ascorbic acid determination in blood plasma. Clin. Chim. Acta, 86: 153-157.

Li, F., Z.Z. Chong and K. Maiese, 2006. Winding through the WNT pathway during cellular development and demise. Histol. Histopathol., 21: 103-124.

Li, J., M.L. Spletter, D.A. Johnson, L.S. Wright, C.N. Svendsen and J.A. Johnson, 2005. Rotenone-induced caspase 9/3-independent and dependent cell death in undifferentiated and differentiated human neural stem cells. J. Neurochem., 92: 462-476.

Li, Z.G., M. Britton, A.A.F. Sima and J.C. Dunbar, 2004. Diabetes enhances apoptosis induced by cerebral ischemia. Life Sci., 76: 249-262.

Lumini-Oliveira, J., A. Ascensao, C.V. Pereira, S. Magalhaes, F. Marques, P.J. Oliveira and J. Magalhaes, 2010. Long-term hyperglycaemia decreases gastrocnemius susceptibility to permeability transition. Eur. J. Clin. Invest., 40: 319-329.

Magni, F., G. Panduri and N. Paolocci, 1994. Hypothermia triggers iron-dependent lipoperoxidative damage in the isolated rat heart. Free Radic. Biol. Med., 16: 465-476.

Maiese, K., Z.Z. Chong and Y.C. Shang, 2007. Mechanistic insights into diabetes mellitus and oxidative stress. Curr. Med. Chem., 14: 1729-1738.

Martinez-Tellez, R., M.J. Gomez-Villalobos and G. Flores, 2005. Alteration in dendritic morphology of cortical neurons in rats with diabetes mellitus induced by streptozotocin. Brain Res., 1048: 108-115.

Maxwell, S.R., H. Thomason, D. Sandler, C. Leguen and M.A. Baxter *et al*., 1997. Antioxidant status in patients with uncomplicated insulin-dependent and non-insulin-dependent diabetes mellitus. Eur. J. Clin. Invest., 27: 484-490.

Mukherjee, P.K., V.L. Marcheselli, C.N. Serhan and N.G. Bazan, 2004. Neuroprotectin D1: A docosahexaenoic acid-derived docosatriene protects human retinal pigment epithelial cells from oxidative stress. Proc. Natl. Acad. Sci. USA., 101: 8491-8496.

Ohkawa, H., N. Ohishi and K. Yagi, 1979. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal. Biochem., 95: 351-358.

Rahimi, R., S. Nikfar, B. Larijani and M. Abdollahi, 2005. A review on the role of antioxidants in the management of diabetes and its complications. Biomed. Pharmacother., 59: 365-373.

Reaven, G.M., L.W. Thompson, D. Nakum and E. Haskins, 1990. Relationship between hyperglycemia and cognitive function in older NIDDM patients. Diabetes Care, 13: 16-21.

Rice-Evans, C. and R. Burdon, 1993. Free radical lipid interactions and their pathological consequence. Prog. Lipid Res., 32: 71-110.

Ryan, C.M. and M. Geckle, 2000. Why is learning and memory dysfunction in type 2 diabetes limited to older adults? Diabet. Metab. Res. Rev., 16: 308-315.

Samiec, P.S., C. Drews-Botsch, E.W. Flagg, J.C. Kurtz, P.J.R. Sternberg, R.L. Reed and D.P. Johns, 1998. Glutathione in human plasma: Decline in association with aging, age-related macular degeneration and diabetes. Free Radic. Biol. Med., 24: 699-704.

Seyrek, K., H. Erbas, M. Serter, C. Yenisey, F. Kiral and H.E. Bardakcioglu, 2010. The effects of dietary ascorbic acid supplementation on collagen and amino acid concentrations in Japanese quails exposed to heat stress. Turk. J. Vet. Anim. Sci., 34: 69-74.

Sharifi, A.M., S.H. Mousavi, M. Farhadi and B. Larijani, 2007. Study of high glucose-induced apoptosis in PC12 cells: role of bax protein. J. Pharmacol. Sci., 104: 258-262.

Siu, A.W. and C.H. To, 2002. Nitric oxide and hydroxyl radical-induced retinal lipid peroxidation *in vitro*. Clin. Exp. Optom., 85: 378-382.

Stadtman, E.R., 1993. Oxidation of free amino acids and amino acid residues in proteins by radiolysis and by metal-catalyzed reactions. Annu. Rev. Biochem., 62: 797-821.

Valko, M., D. Leibfritz, J. Moncol, M.T. Cronin, M. Mazur and J. Telser, 2007. Free radicals and antioxidants in normal physiological functions and human disease. Int. J. Biochem. Cell Biol., 39: 44-84.

Vincent, A.M. and K. Maiese, 1999. Nitric oxide induction of neuronal endonuclease activity in programmed cell death. Exp. Cell Res., 246: 290-300.

Yenisey, C., K. Seyrek, M. Serter, F. Kargin-Kiral and H.E. Bardakcioglu, 2006. Effects of dietary vitamin c supplementation on glutathione, malondialdehyde and nitric oxide concentrations in brain and heart of laying Japanese quails exposed to heat stress (34.8°C). Rev. Med. Vet., 157: 149-152.