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Reduction of diabetes-induced oxidative stress by phosphodiesterase inhibitors in rats

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Abstract

Increased oxidative stress has been suggested to be involved in the pathogenesis and progression of diabetic tissue damage. The aim of this study was to investigate the effect of different phosphodiesterase inhibitors on lipid peroxidation and total antioxidant capacity (TAC) of plasma in streptozotocin-induced diabetic rats (*Rattus norvegicus*). Rats became diabetic by a single administration of streptozotocin (STZ, 45 mg/kg). The effects of 15-days treatment by milrinone, sildenafil, and theophylline as cyclic-AMP and -GMP phosphodiesterase inhibitors (PDEIs) on diabetes-induced oxidative stress were studied. The levels of glucose, malonedialdehyde (MDA) the by product of lipid peroxides, and TAC (FRAP test) were estimated in plasma of control and experimental groups of rats. A significant increase in the levels of plasma glucose, and MDA and a concomitant decrease in the levels of TAC were observed in diabetic rats. These alterations were reverted back to near normal level after the treatment with PDEIs. Treatment of diabetic rats by PDEIs reduced MDA levels and increased TAC in the order of milrinone > sildenafil > theophylline. In conclusion, the present investigation show that PDIS possesses antioxidant activities, which may be attributed to their enhancing effect on cellular cyclic nucleotides contributing to the protection against oxidative stress in streptozotocin-induced diabetes. Exact mechanism of protective actions of cAMP- and cGMP-phosphodiesterase remains to be elucidated by further studies. This finding may suggest a place for PDEIs in maintaining health in diabetes.

Keywords: Oxidative stress; Diabetes; Phosphodiesterase inhibitors; Sildenafil; Milrinone; Theophylline

1. Introduction

Free radicals are continually produced in the body as a result of normal metabolic processes and interaction with environmental stimuli. Oxidative stress results from an imbalance between radical-generating and radical-scavenging systems, i.e. increased free radical production or reduced activity of antioxidant defenses or both. Oxidative stress is currently suggested as the mechanism underlying diabetes and diabetic complications (Halliwell and Gutteridge, 1989). Enhanced oxidative stress and changes in antioxidant capacity, observed in both clinical and experimental diabetes mellitus are thought to be the etiology of chronic diabetic complications (Baynes, 1991). Many of the complications of diabetes including retinopathy and atherosclerotic vascular disease, the leading cause of mortality in diabetes have been linked to oxidative stress and antioxidants (i.e. vitamin E, C,...) have been considered as treatments (Ceriello, 2000; Cunningham, 1998; Reaven et al., 1995; Strain, 1991; Young et al., 1991).

The phosphodiesterases (PDEs) are a superfamily of enzymes which catalyse the hydrolysis of the cyclic nucleotides cAMP and cGMP to their corresponding inactive 5-monophosphate counterparts. The cyclic nucleotides play a prominent role in the regulation of important cellular functions and PDE inhibition can therefore elicit a variety of effects (Dal-Piaz and Giovannoni, 2000). At present at least 11 different families of PDE isoenzymes are known. Some of them are characterized by substrate specificity (cAMP or

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cGMP), different kinetic properties and different tissue distribution. The xanthine analogue theophylline has been used as a cAMP-PDEI in the treatment of asthma for more than 60 years. Milrinone is a non-selective PDEI which increase both cAMP and cGMP levels and is used in cardiovascular diseases as an inotropic agent. Sildenafil is a selective cGMP-PDEI inhibitor used in the treatment of sexual dysfunctions (Souness et al., 2000). There are experimental evidences that increasing intracellular cAMP and cGMP by use of PDEI like theophylline and sildenafil prevent from induction of oxidative stress in salivary glands (Abdollahi et al., 2003a,b). There are also good evidences on anti lipid-peroxidation properties of either cAMP or cGMP analogs in rat neural (Keller et al., 1998), renal cells (Kohda and Gemba, 2001), lung (Sciuto et al., 1997; Zhang et al., 1999), and sperm function (Zhang et al., 1998). The relationship with endothelium dysfunction in diabetes patients and low cGMP levels has been also reported (Aydin et al., 2001).

This study was undertaken because to our knowledge there were no published reports on the effects of PDEIs on streptozotocin-induced diabetes mellitus in rats.

2. Materials and methods

2.1. Chemicals

Hydrogen peroxide, sodium sulfate, Triton X-100, 2,4,6-tripyridyl-*s*-triazine (TPTZ), 2-thiobarbituric acid (TBA), trichloroacetic acid (TCA), Tris base, phosphate buffer, *n*-butanol from Merck (Germany), and glucose oxidase, peroxidase, *o*-dianisidine–HCl, 1,1,3,3-tetraethoxypropan, and streptozotocin from Sigma-Aldrich (USA) were used in this study.

2.2. Animals

Male Wistar rats (*Rattus norvegicus*; from Animal House of Faculty of Pharmacy, TUMS) weighing 185–230 g were used in this study. The animals were housed under controlled environmental conditions of temperature (22 ± 2 °C) and a 12-h light/dark cycle. Upon arrival, the animals were allowed to acclimatize for 7 days. They were fed with standard rat chow (Dampars, Iran). No restriction was for access of animals to food and water. Animals were weighed and the extent of their intake of food and water consumption were monitored daily. The protocol of study was approved by ethical committee of TUMS.

2.3. Induction of diabetes

After acclimatization, rats were subjected to a 16-h fast. The fasting blood glucose concentrations were measured from the tail vein. Diabetes was induced in rats with STZ at a dose of 45 mg/kg intraperitoneally (Ito et al., 2001). The STZ was freshly dissolved in citrate buffer (0.01 mol/L, pH 4.5). Control rats received only the buffer. Thirty-six hours after injection of streptozotocin, diabetes was confirmed in STZ-treated rats with fasting blood glucose concentrations between 10 and 14 mmol/L.

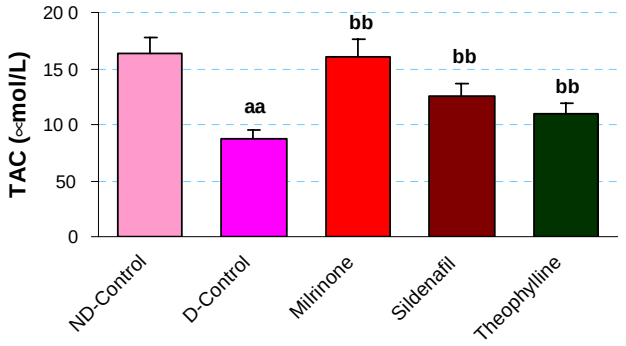

Fig. 1. Effects of phosphodiesterase inhibitors on plasma total antioxidant capacity (TAC) in diabetic rats. Diabetes was induced by administration of streptozotocin (freshly dissolved in citrate buffer 0.01 mol/L, pH 4.5, 45 mg/kg intraperitoneally) to drug-treated and diabetic control (D-Control) rats. Non-diabetic control (ND-Control) animals received only the buffer. Milrinone, sildenafil, and theophylline were dissolved in drinking water at doses of 3.17, 1, and 100 (mg/kg/day), respectively for 15 successive days. Data are means ± SEM of 6 animals in each group. [aa]Difference between ND-Control and D-Control values is significant at $P < 0.01$. [bb]Difference between D-Control and treated groups is significant at $P < 0.01$.

2.4. Treatment protocol

Diabetic animals were divided into four groups of 6 rats each. The first group assigned as diabetic control (normal drinking water), and three remaining groups received milrinone (3.17 mg/kg/day), sildenafil (1 mg/kg/day), and theophylline (100 mg/kg/day) for 15 successive days via drinking water. Rats in all groups were sacrificed after 15 days. Another group of animals was assigned as non-diabetic control and were not received STZ. The body weights of the experimental animals were taken daily to ensure good health in accordance.

At the end of 15 days, after blood sampling, animals were sacrificed by decapitation. No significant difference in food intake, water consumption, and body mass were recorded among control and treated groups during the treatment.

2.5. Glucose measurement

Glucose concentration in plasma samples was measured in the presence of glucose oxidase and peroxidase using *o*-dianisidine–HCl as a chromogen. The amount of glucose formed is related to amount of *o*-dianisidine oxidation products that measured spectrophotometrically at 436 nm.

2.6. Thiobarbituric acid reactive substances (TBARS) measurement

Plasma samples were mixed with trichloroacetic acid (20%) and the precipitate was dispersed in H_2SO_4 (0.05 M).

TBA (0.2% in 2 M sodium sulfate) was added and heated for 30 min in boiling water bath. TBARS adducts were extracted by *n*-butanol and absorbance was measured at 532 nm (Satho, 1978).

2.7. Ferric reducing ability of plasma (FRAP) assay

Total antioxidant capacity of blood was determined by measuring the ability of plasma to reduce Fe^{3+} to Fe^{2+}. The complex between $Fe^{2+}$and TPTZ gives a blue color with absorbance at 593 nm (Iris et al., 1999).

2.8. Statistical analysis

The results were analyzed for statistical significance by one-way ANOVA test and Tukey's post hoc multicomparison. All data were expressed as means ± SEM of 6 animals in each group. Differences between groups were considered significant at $P<0.05$.

3. Results

Plasma glucose of control animals was 5.1 ± 0.1 which reached 21.8 ± 0.8 mmol/L in STZ-induced diabetes at the end of the study period. Plasma glucose level was not affected by PDEI treatment. The mean TAC and TBARS in control animals were 163 ± 14.67 and 1.45 ± 0.13 μmol/L, respectively, reaching 88 ± 7.92 and 2.33 ± 0.21 μmol/L respectively in diabetic rats ($P<0.01$) (Fig. 1). Treatment of diabetic rats with milrinone (1.55 ± 0.14 μmol/L), sildenafil (1.75 ± 0.16 μmol/L), and theophylline (1.82 ± 0.16 μmol/L) decreased plasma TBARS significantly ($P<0.01$, Fig. 2).

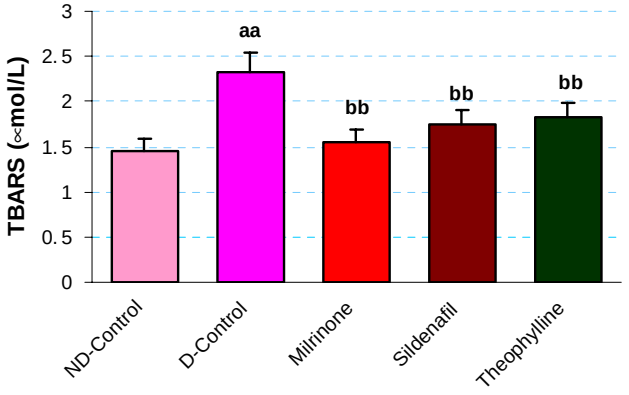

Fig. 2. Effects of phosphodiesterase inhibitors on plasma lipid peroxidation in diabetic rats. Diabetes was induced by administration of streptozotocin (freshly dissolved in citrate buffer 0.01 mol/L, pH 4.5, 45 mg/kg intraperitoneally) to drug-treated and diabetic control (D-Control) rats. Non-diabetic control (ND-Control) animals received only the buffer. Milrinone, sildenafil, and theophylline were dissolved in drinking water at doses of 3.17, 1, and 100 (mg/kg/day) respectively for 15 successive days. Data are means ± SEM of 6 animals in each group. [aa]Difference between ND-Control and D-Control values are significant at $P<0.01$. [bb]Difference between D-Control and treated groups is significant at $P<0.01$.

4. Discussion

Data of this study provide a good indication of the presence of oxidative stress in blood of diabetic rats which is well supported by previous reports and suggests that free radicals play a crucial role in the STZ-induced diabetes (Maritim et al., 2003; Anwar and Meki, 2003). Reduced antioxidant levels as a result of increased free radical production in experimental diabetes have been reported by many authors (Grankvist et al., 1981; Saxena et al., 1993; Giugliano et al., 1995). Damasceno et al. (2002) and Gul et al. (2002) reported that STZ produces oxidative stress and depletion of antioxidant systems in both blood and tissues. Moreover, Kedziora et al. (2002) found a reduction in antioxidant defense and elevation in lipid peroxidation in kidney of STZ-induced diabetic rats. Increased lipid peroxidation suggests an increase in reactive oxygen species, which could be due to increased glucose concentration (Hunt et al., 1990). The exact mechanism by which increased blood glucose concentrations lead to lipid peroxidation in plasma and erythrocytes of diabetic patients is not known. However, in vitro studies suggest that enolization of glucose under physiological conditions results in reduction of molecular oxygen and production of oxygen free radicals and α-ketoaldehydes, which ultimately cause peroxidative breakdown of phospholipids in erythrocyte membranes and accumulation of malondialdehyde, a terminal compound of lipid peroxidation (Akkus et al., 1996). In explanation of reduced TAC, there are several reports on reductions in the activities of most important antioxidant enzymes like superoxide dismutase (Sozmen et al., 1999; Andallu and Varadacharyulu, 2003), catalase (Bhatia et al., 2003), and glutathione peroxidase (Bhatia et al., 2003; Sekeroglu et al., 2000) in diabetes.

However, treatment of the diabetic rats with PDEIs decreased to some extent plasma TBARS and increased TAC. The antioxidant effect of milrinone was better than sildenafil and theophylline which caused dramatic elevation in TAC and reduced oxidative stress. This suggests a protective role for PDEIs, which could be due to their antioxidative effects. The pharmacologic actions of PDEIs back to their potential to increase intracellular cAMP and cGMP. The regulation of cell function by extrinsic factors occurs through a series of second-messenger signals that are initiated by ligand-receptor interaction and then modulate both the intensity and the nature of immediate and delayed cellular responses. In this manner, second messengers exert control over principal cellular events such as metabolism, secretion, structure, and replication. There is evidence that biological responses triggered by oxidative products are associated with lipid peroxidation derivatives, which are able to induce various pathogenic intracellular signals involving calcium, G-proteins, cAMP, cGMP, phospholipase C and D, protein kinase C, ceramide, and MAP kinase cascade leading to cellular dysfunction (Leonarduzzi et al., 2000).

Thus increasing cyclic nucleotides by use of PDEIs could overcome to oxidative stress-induced cellular dysfunctions and apoptosis. Supporting this conclusion, Polte and Schroder (1998) reported an antioxidant property for nitric oxide (NO) donors in vascular endothelium through concerted action of cGMP and cAMP. They showed that *S*-nitroso-*N*-acetyl-D,L-penicillamine (SNAP) protects from TNF-mediated endothelial cell toxicity. The cytoprotection by SNAP was completely abolished by the adenylyl cyclase inhibitor 2,5-dideoxyadenosine and mimicked by 8-bromo cAMP or forskolin. SNAP produced significant increases in cGMP and cAMP, both being abrogated in the presence of the NO scavenger 2-phenyl-4,4,5,5,-tetramethylimidazoline-1-oxyl-3-oxide (PTIO). They concluded a crucial role for cAMP in mediating NO-induced endothelial protection against TNF, possibly through cGMP-dependent inhibition of cAMP breakdown. Therefore, NO-dependent endothelial protection may ultimately result from cAMP-induced up-regulation of antioxidant proteins or down-regulation of cytotoxic processes. The antioxidant property and cytoprotective functions of NO through concerted action of cyclic nucleotides has found many supports in the recent years. NO was reported to afford protection against cellular damage induced by oxidative stress in lung fibroblasts (Wink et al., 1993), vascular endothelium (Motterlini et al., 1996; Polte et al., 1997a,b), human neuroblastoma cells (Andoh et al., 2003), and salivary glands (Abdollahi et al., 2000a,b; Abdollahi and Safarhamidi, 2002). In addition, previous investigations from our laboratory provided evidence that cAMP/cGMP PDEIs themselves induce protection against salivary gland oxidant injury caused by heavy metals (Abdollahi et al., 2003a,b).

The relationships between sperm lipid peroxidation or cyclic nucleotides and sperm motility in normospermic and asthenozoospermic specimens have also been reported suggesting the protective roles of cyclic nucleotides against oxidative stress (Leonarduzzi et al., 2000). Moreover dipyridamole a cAMP/cGMP PDEI (Abdollahi et al., 2003c; Fawcett et al., 2000) when tested in diabetic nephropathy reduced oxidative stress and enhanced expression of NO synthase (NOS) gene via cAMP-dependent protein kinase pathway and possibly scavenging the superoxide anion by nNOS and eNOS (Onozato et al., 2003; Iimura et al., 1996; Michell et al., 2001). Thus the second mechanism for the actions of PDEIs could be activation NO system against oxidative stress through overexpression of NOS. Supporting this mechanism, there is evidence that concentration of total NO is increased in response to oxidative stress as a consequence of illnesses like inflammatory bowel disease (Jahanshahi et al., 2005) and diabetes (Astaneie et al., 2005) in human. This means that increasing of NO might have a protective role in reduction of oxidative stress but its mechanism of action is still unclear.

In conclusion, the present investigation show that PDEIs possesses antioxidant activities, which may be attributed to their enhancing effect on cellular cyclic nucleotides antioxidant defense contributing to the protection against oxidative damage in streptozotocin diabetes. This finding may suggest a place for PDEIs in maintaining health in diabetes. Exact mechanism of protective actions of cAMP- and cGMP-phosphodiesterase remains to be elucidated by further studies.

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