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Pancreatic O^6-Methylguanine DNA Methyltransferase Level in Streptozotocin-Induced Diabetic Rats

Yıldız Dinçer[1]*, Ufuk Çakatay[2], Refik Kayalı[1], İlker A. Yılmaz[1], Ayşegül Telci[2], Nilgün Çelebi[3], Hüsrev Hatemi[4], Nazif Bağrıaçık[4] and Tülay Akçay[1]

[1] İstanbul University, Cerrahpaşa Medical Faculty, Department of Biochemistry, İstanbul, 34300-Turkey.

[2] İstanbul University Medical School, Central Laboratory of Clinical Biochemistry, Çapa, İstanbul, 34390-Turkey.

[3] Çekmece Nuclear Research Center, İstanbul 34461-Turkey.

[4] İstanbul University, Cerrahpaşa Medical Faculty, Department of Endocrine and Metabolism, İstanbul, 34300-Turkey.

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ABSTRACT

O^6-methylguanine which is formed in cellular DNA by alkylating agents is a toxic and mutagenic lesion. O^6-methylguanine is repaired by DNA repair protein, O^6-methylguanine DNA methyltransferase. Deficient repair of O^6-methylguanine has been suggested to be a contributory factor in the etiology of some diseases. Streptozotocin (SZ) is an alkylating compound that is often used to induce diabetes in experimental animals. We aimed to determine O^6-methylguanine DNA methyltransferase level in pancreas of SZ-induced diabetic rats. Serum levels of glucose and fructosamine, and pancreatic O^6-methylguanine DNA methyltransferase level were measured in the streptozotocin-induced diabetic and control rats. Serum glucose and fructosamine levels were found to be higher in the diabetic group than those in the control group ($P<0.001$ and $P<0.001$, respectively). Pancreatic O^6-methylguanine DNA methyltransferase level was lower in the diabetic group (68.06 ± 12.37 fmol/mg protein) than in the control group (89.45 ± 13.83 fmol/mg protein), ($P<0.01$) and there was a negative correlation between serum fructosamine level and pancreatic O^6-methylguanine DNA methyltransferase level (r: -0.6, $P<0.02$). These data imply that O^6-methylguanine DNA methyltransferase depletion may be one of the mechanisms for diabetogenic action of SZ in rats.

SZ, an antibiotic isolated from streptomyces achromogenes, is widely used for the induction of diabetes in experimental animals, due to damaging action on the pancreatic β cells which causes cell death (28). Damage to DNA of pancreas β-cells by SZ is caused by known two major pathways. The first one arising from the NAD^+ depleting action of SZ. SZ induces DNA strand breakage in β-cells (29, 16). Excessive DNA strand breakage activates poly-(ADP-ribose) polymerase (PARP, EC 2.4.2.30) in pancreatic cells which leads to NAD^+ depletion by catalyzing the formation of poly-(ADP-ribose) from NAD^+ (29, 26). NAD^+ is an important cofactor in energy metabolism, and its depletion results in lower ATP production. Thus cells can die from energy loss (32). The other arises from radical metabolites of SZ (23). Methyl carbonium ion, which is produced from the methylated nitrosourea moiety during decomposition of SZ may damage DNA by alkylation (1, 15). Alkylating agents lead to DNA damage by transfering alkyl group to DNA bases. They react at many sites in DNA, predominantly producing 7-methylguanine, 3-methyladenine, and O^6-methylguanine (O^6-MG). The most harmful lesion is O^6-MG because of its toxicity and mutagenicity. O^6-MG adducts on DNA lead to the formation

*Correspondence to: Yıldız Dinçer, Cebesoy Sok., Eko Tek, B Blok No: 5/3 Sahrayı Cedid, Erenköy, İstanbul - Turkey
Tel: +90 216 3857508, Fax: +90 212 2726730
E-mail: stare63@yahoo.com

of stable cytotoxic DNA crosslinks. Therefore methylation of guanines by SZ induces apoptosis in cells (23). Moreover, O^6-MG pairs with thymine, in addition to cytidine, during DNA replication, causing G ≡C →A=T transition mutation (13). O^6-MG is repaired in mammalian cells by O^6-methylguanine DNA methyltransferase O^6-MGMT (E. C 2.1.1.63), a DNA repair enzyme. The methyl group at the O-6 position of guanine is transferred irreversibly to a cysteine residue within O^6-MGMT, thus inactivating the enzyme (25). Restoration of native O^6-MGMT requires *de-novo* protein synthesis. Therefore, in absence of new O^6-MGMT synthesis, a cell contains a finite capacity for removal of O^6-MG by O^6-MGMT (19). Defective repair of O^6-MG has been shown in the etiology of some diseases (11, 9). The purpose of the present study was to determine pancreatic O^6-MGMT level in SZ-induced diabetic rats, therefore to examine whether O^6-MGMT plays a role on the development of experimental diabetes.

MATERIAL AND METHODS

Male Wistar rats with a mean weight of 230 g, aged 8 weeks, and supplied by Center for Experimental and Applied Medical Research, University of İstanbul, Turkey were used in this study. Animals were housed in conventional wire-mesh cages, and were fed a standard laboratory diet. Rats were randomly divided into two groups. Diabetes was induced by using a single intravenous injection of 60 mg/kg body weight SZ (Sigma, St Louis, MO USA), (n: 17). Rats not pretreated with SZ, which formed the control group (n: 14), were given only saline by intravenous route. After 5 weeks, rats were sacrified under ether anaesthesia and their blood were collected for the colorimetrical measurement of serum glucose and fructosamine levels on the DAX-72 discrete auto analyzer. The results were used as an indication of diabetes. Pancreas was removed and chilled immediately. An aliquot of the pancreas was rinsed and homogenized in three volumes of ice-cold buffer composed of 50 mM Tris (pH 7.5), 1 mM EDTA, 10 mM DTT, 0.2% Triton X-100, followed by centrifugation at 12000 × *g* for 10 min at 4 °C. Supernatant was used as cellular extract containing enzyme activity. O^6-MGMT level was assayed by measuring the disappearance of O^6-methyl guanine from methylated DNA (3). Alkylated micrococcus lysodeikticus DNA by ^{3}H-methyl nitrosourea was used as substrate for O^6-MGMT. O^6-MGMT activity was measured by scintillation counting. Results were expressed as femto mol CH_3 removed from DNA per mg protein (fmol/mg protein). Protein concentration of homogenate was measured with the folin phenol reagent (17).

Significant differences between two groups were carried out by using the Non-parametric Mann-Whitney U test. Data are given mean±SD. When not specified, $P<0.05$ was considered significant. Correlation analysis was performed by using Spearman correlation coefficient.

RESULTS

Serum glucose and fructosamine levels of the both groups are given in Table 1. Serum glucose ($P<0.001$) and fructosamine ($P<0.001$) levels in diabetic rats were higher than those in the control group. Pancreatic O^6-MGMT level was determined in 17 diabetic and 14 non-diabetic rats. O^6-MGMT level in the diabetic group was significantly lower than in the control group ($P<0.02$) (Fig. 1). There was a significant negative correlation between pancreatic O^6-MGMT level and serum fructosamine level (r: −0.6, $P<0.02$).

Table 1 *Serum glucose and fructosamine levels of both the diabetic and control rats (Mean±SD)*

	Control group (n:14)	Diabetic group (n:17)
Serum glucose (mmol/L)	9.83±1.75	26.88±6.00*
Serum fructosamine (mmol/L)	1.05±0.07	2.50±0.74*

*$P<0.001$ versus Control group.

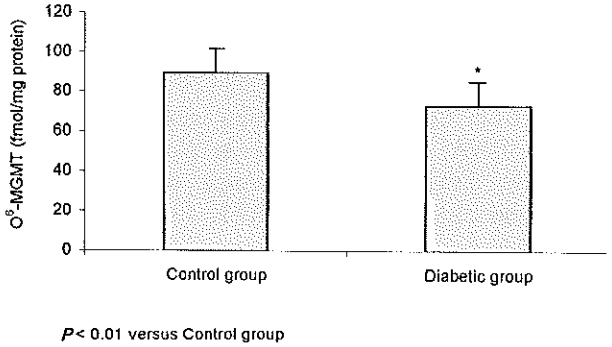


Fig. 1 Pancreatic O^6-MGMT level of control and diabetic rats

DISCUSSION

SZ exerts its diabetogenic action by different mechanisms simultaneously. NAD-depleting action of SZ on the β cells is well documented and has led to the suggestion that low intracellular levels of NAD^+ cause severe lesions of pancreatic cell metabolism leading to cell death (29, 26, 23). On the other hand, SZ is a strong alkylating agent. In an early study, it was observed that following administration of SZ, labelled in the methyl group, the radioactivity preferentially accumulated in the pancreas and in association with nucleic acids (1, 21). Recently Murata *et al.* (23) suggested that SZ induces DNA damage by methylation of guanine via methyl cations and, this alkylation may be responsible for triggering apoptosis and subsequently diabetes. Nelson *et al.* (24) was determined increased O^6-MGMT mRNA level in cultured β cells within 12–24 h after the treatment with methylnitrosourea, an alkylating agent, but no increase in O^6-MGMT level was observed 24 or 48 h after exposure. They suggested that newly formed O^6-MGMT may have been consumed in the repair process very rapidly, thus no increase in O^6-MGMT level was found. As far as we know, there is no report examining O^6-MGMT level in the pancreas of SZ-induced diabetic rats. In the present study O^6-MGMT level was found to be lower in the diabetic rats than in the controls. This was a preliminary study for repair of O^6-MG in diabetic rats. Determined decrease in the present study can be evaluated as a result of the consumption of O^6-MGMT for repair of O^6-MG adducts on DNA formed by methyl carbonium ions derived from SZ. However, inactivation of O^6-MGMT gene by promoter hypermethylation has been shown (7). β cells contain a finite level of O^6-MGMT might be more susceptible to SZ when O^6-MGMT is completely depleted due to increased consumption and/or gene inactivation.

Other alkylation products, 7-methylguanine and 3-methyladenine, are just toxic lesions. They have no mutagenic capacity, and are removed by alkylpurine-DNA-N-glycosylase (APNG), leaving and apurinic/apyrimidinic (AP) site that is acted upon by an AP endonuclease. The resulting DNA strand breaks activate PARP. In mice lacking APNG, PARP activation and β cell necrosis was markedly attenuated after a single dose of SZ (5). It has been shown that PARP knockout mice are resistant to the development of diabetes induced by SZ (26, 4, 22). However treatment of rats with both SZ and PARP inhibitors led to the development of tumors of the β cells in a few animals (10). This indicates the protective mechanism of cells against mutagenesis: excessive PARP activation and severe NAD^+ depletion may help the organism by killing cells with excessive DNA damage, so minimizing the occurrence of harmful mutations.

On the other hand, tumor cells with high level of O^6-MGMT exhibit resistance to nitrosourea-derived antitumor agents (15, 25). They exert their antitumor actions by forming stable interstrand cross-links on DNA which block replication (18). SZ was suggested as a DNA-methylating agent which depletes O^6-MGMT and sensitizes nitrosourea-resistant cell lines (27, 20). However, as far as we know, there is no report indicating O^6-MGMT depleting action of SZ as a probable mechanism on the pathogenesis of diabetes. In the present study, determined negative correlation between pancreatic O^6-MGMT level and serum fructosamine level which is used as indicator of diabetes, emphasizes the importance of O^6-MGMT depletion on onset and/or progression of diabetes mellitus.

Altered enzymic activity by non-enzymic glycation of enzymes is well documented (8, 30). Nonenzymatic glycation is a spontaneous chemical reaction between glucose and the amino groups of proteins in which reversible Shiff bases and more stable Amadori products are formed. Advanced glycation end produts (AGEs) are then formed through oxidative reactions and cause irriversible chemical modifications of proteins which can lead the alterations in their structures and functions (31). Although there is no evidence for inactivation of O^6-MGMT by high level of glucose, this also may explain decreased O^6-MGMT level in SZ-induced diabetic rats, presented here. Further investigations are needed to clarify this issue.

There are considerable data suggesting that alkylating agents may contribute to an incrased prevalence of diabetes (12, 6, 14, 2). In this preliminary study, decreased O^6-MGMT level was determined in streptozotocin-induced diabetic rats. Apart from the present study, likely, we have determined low level of O^6-MGMT in leukocytes from type 1 and type 2 diabetic patients as compared to their respective controls (unpublished data). Data imply that O^6-MGMT depletion may be one of the mechanisms for diabetogenic action of SZ in rats. However, it is highly new suggestion and it has not been published so far. A more detailed study on the molecular basis is the goal of our next investigation.

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