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Possible protective effect of procainamide as an epigenetic modifying agent in experimentally induced type 2 diabetes mellitus in rats

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Abstract *Background:* Diabetes mellitus (DM) is a metabolic disease that is associated with disturbed carbohydrates, lipids and protein metabolism due to insulin deficiency and/or impaired insulin action. Recently epigenetic mechanisms were shown to be involved in endocrine cell differentiation and islets function. Procainamide which is a cardiac antiarrhythmic drug has been recently classified as one of the epigenetic drugs targeting DNA methylation.
Aim: The present study was designed to evaluate the effect of procainamide as a demethylating epigenetic agent on streptozotocin-induced type 2 diabetes mellitus in rats.
Methods: Fifty adult male albino rats of weight ranging from 150 to 200 g were included in this study. Rats were divided into five groups (each of 10 rats) as follows: group I: served as a normal control group, group II: diabetic rats that received 1 ml gum acacia 2% orally, daily for 4 weeks, group III: diabetic rats that received procainamide (20 mg/kg body weight)/day, orally for 4 weeks, group IV: diabetic rats that received metformin (300 mg/kg/day), orally for 4 weeks, group V: diabetic rats that received both procainamide and metformin in the same previous doses for 4 weeks. The following parameters were assessed in rats of all studied groups: fasting blood glucose level, serum insulin level, serum tumor necrosis factor alpha (TNF-α) (as a proinflammatory cytokine as well as an indirect biomarker of DNA methylation) and DNA methyltransferase enzyme (DNMT) activity in pancreatic tissues (as a direct marker of DNA methylation).
Results: The present study revealed that combined administration of both procainamide and metformin produced a statistically significant reduction of fasting blood glucose levels as compared to untreated diabetic rats as well as diabetic rats treated by either procainamide or metformin alone. In

Abbreviations: CpG, cytosine–phosphate–guanine; DM, diabetes mellitus; DNA, deoxyribonucleic acid; DNMT, DNA methyltransferase; TNF-α, tumor necrosis factor-alpha.

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addition, procainamide administration either alone or in combination with metformin resulted in a statistically significant rise of serum insulin levels. TNF-α levels were statistically elevated in diabetic untreated rats as well as those treated with metformin only while procainamide intake led to its statistical decrease. Also, procainamide administration produced a statistically significant reduction in the activity of DNA methyltransferase in pancreatic tissues reflecting its possible role as a demethylating agent that increases insulin expression and release by pancreatic cells.

Conclusion: The present work could provide a proof of concept that procainamide could be used as a possible therapeutic potential in type 2 diabetics as an epigenetic demethylating agent to increase insulin levels and it is better to be used in combination with oral hypoglycemic agent e.g. metformin to decrease insulin resistance.

1. Introduction

Diabetes mellitus (DM) is a group of metabolic diseases characterized by hyperglycemia resulting from defect in insulin secretion, insulin action or both and it is associated with disturbed carbohydrates, lipids and protein metabolism.[1] DM can be classified according to the treatment required to control it into insulin dependent (type 1) and non-insulin dependent (type 2) diabetes or it may be classified according to the age at which the disease develops into juvenile or maturity onset diabetes. Pathogenesis of DM represents a complex of interaction between metabolic, genetic and environmental factors as well as inflammatory mediators.[2]

Evidence are emerging that several diseases and behavioral pathologies result from defects in gene functions. The best studied example is cancer but other diseases such as autoimmune diseases, asthma, type 2 diabetes and metabolic disorders display aberrant gene expression. Gene function may be altered by either change in the sequence of DNA or change in epigenetic programming.[3]

Epigenetic information is defined as heritable information other than the DNA sequence that is critical to the proper functioning of the gene .These epigenetic alterations are potentially reversible and DNA methylation represents the most characterized epigenetic modification. It is represented by methylation of cytosine residues within the context of the cytosine–phosphate–guanine (CpG) dinucleotide which is catalyzed by enzymes belonging to the DNA methyltransferases family (DNMTs). Although the density of CpG sites in the genome is very low, there are clusters of CpG sites, known as 'CpG islands', which are generally kept unmethylated. The CpG islands remain free of methylation and are associated with transcriptionally active genes, predominantly so-called 'housekeeping' genes. When a CpG island in the promoter region of a gene is methylated, expression of the gene is repressed.[4]

With epigenetic drugs, it is possible to reverse aberrant gene expression profiles associated with different disease states and several epigenetic drugs targeting DNA methylation has been tested in clinical trials. Understanding the epigenetic machinery and the differentiation roles of its components in specific disease states is essential for developing targeted epigenetic therapy.[5]

Recently, epigenetic mechanisms were shown to be involved in pancreatic endocrine cell differentiation and islet function. In depth understanding of epigenetic landscape can help to improve protocols to enhance pancreatic beta cells proliferation and lead to the discovery of novel therapeutic agents.[6]

Inhibitors of DNA methyltransferases may be either nucleoside analogs such as 5-aza-2′deoxycytidine, decetabine and zebularine or non-nucleoside such as procainamide and hydralazine. Procainamide is a cardiac antiarrhythmic drug that has been approved for treatment of life threatening ventricular arrhythmias. Recently it has been classified as one of DNA demethylating epigenetic agents with growth inhibitory effects in human cancer cells.[7] Therefore, the present study aimed to assess the possible protective effect of procainamide as a demethylating epigenetic agent on experimentally induced type 2 DM in rats.

2. Material and methods

This study was carried out on fifty adult male albino rats of body weight ranging from 150 to 200 g. They were housed under the same environmental conditions of light and temperature, fed standard diet and had free access to water. The study protocol was approved by ethics committee of Faculty of Medicine, Alexandria University.

The animals were divided into five groups (each of 10 rats) as follows:-

Group I (normal control group): This group received 1 ml of 0.1 M sodium citrate buffer (pH 4.5) intraperitoneally (i.p.).

The remaining 40 rats were subjected to induction of type 2 DM by a single i.p. injection of 40 mg/kg body weight streptozotocin (STZ) (Sigma Chemical Co., Switzerland) after being dissolved in 0.1 M sodium citrate buffer (pH 4.5).[8] One week after STZ injection, rats were assessed for hyperglycemia.[9]

When DM was developed, rats were randomly assigned into the following groups:

Group II (diabetic untreated group): This group included rats with induced DM that received 1 ml of 2% gum acacia/day (Arabic Laboratory Equipment Co.) orally for 4 weeks.

Group III (procainamide-treated diabetic group): This group included diabetic rats that were given procainamide orally for 4 weeks in a daily dose of 20 mg/kg body weight.[10]

Group IV (metformin-treated diabetic group): This group involved diabetic rats that received metformin (as oral anti-diabetic drug of biguanide class that decreases insulin resistance) in a daily dose of 300 mg/kg body weight orally for 4 weeks.[11]

Group V (procainamide- and metformin-treated diabetic group): Rats of this group were given both procainamide and