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Research Article

Pathophysiological mechanisms of type 2 diabetes mellitus and the effects of metformin treatment in adult male albino rats.

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Abstract

Background: Type 2 diabetes mellitus (T2DM) is a chronic global disease characterized by serious complications. Evidence has shown that free radicals' formation is involved in the pathogenesis of the disease and development of its complications. T2DM is associated with disrupted glucose and lipid homeostasis with abnormal insulin sensitivity and/or secretion. No single treatment regimen is absolutely 100% effective. Metformin is a first-line treatment for T2DM whose main actions are the suppression of gluconeogenesis and the improvement of glucose uptake and insulin sensitivity. However, the exact mechanism of action is not clearly understood. Therefore, the current study aimed to assess the protective effects of metformin against diabetic complications in rats with explanation of the possible underlying mechanisms. **Methods:** rats were allocated in 3 groups. group1: control (C) received normal pellet diet (NPD), group2: diabetic (D) received high fat diet (HFD) for 2 weeks then injected intra-peritoneally (IP) with single low dose of streptozotocin (STZ) (30 mg/kg), group3: diabetic metformin treated (DM) received metformin 500 mg/kg/day by gavage for 4 weeks. Rats were sacrificed after 4 weeks of treatment. **Results:** The damaging effect of STZ on the pancreatic beta cells resulted in T2DM that was evaluated by assessment of glucose, insulin, Homeostasis model assessment of insulin resistance (HOMA-IR), TGs, LDL, HDL, Urea, and malodialdehyde (MDA) in the diabetic rats 'serum. **Conclusion:** The results suggested the protective effects of metformin on ameliorating the diabetic effects.

Keywords: Antidiabetic drugs, Metformin, Insulin resistance, DM

Introduction

Diabetes mellitus is a worldwide metabolic disorder characterized by hyperglycemia with subsequent insulin resistance. It is expected to increase more and more in the next years according to the International Diabetes Federation, (2017). It has serious micro and macro-vascular complications. Since no single medication is absolutely effective, studying the pathophysiological mechanisms of the disease may open the way for new medications to develop and help in attenuating the progression of the disease and its possible complications [1, 2]. Metformin (MET) is one of the oral antidiabetic drugs commonly used in Type2 diabetes (T2DM) [3]. However, because of the multifactorial mechanisms of the disease and its complications, the therapeutic effects of MET have to be reconsidered and its efficacy reevaluated. An example of an experimentally induced animal model of T2DM is the HFD/STZ rat model. This model involves a combination of a diet rich in fat content with low dose of the beta cell cytotoxic drug (STZ), which results in a decline in the functional mass of the pancreatic beta cells. This animal model mimics the natural progression from insulin resistance to frank hyperglycemia and T2DM in a short time [4]. The

aim of the present work is to induce Type2 DM in adult male albino rats and to study in them some pathophysiological changes that lead to the disease and its complications and the effect of MET treatment on these changes.

Materials and methods

Animals

Twenty-four healthy adult male albino rats of local strain were obtained from Egyptian Organization for Biological Products and Vaccines (Cairo, Egypt). The average body weight at the start of the experiment was 197.5±14.75 gram. They were left for 2 weeks acclimatization on NPD and housed in well ventilated cages made of polypropylene with dimensions 30 × 20 × 13 cm as 4 rats/cage in natural dark light cycles. The protocol of work was approved by the Animal Care and Use Committee of Faculty of Medicine, Minia University, Egypt. No.53/6:2021. Date 14 June 2021.

Chemicals

Streptozotocin (STZ) was purchased in the form of powder from Sigma-Aldrich chemical company, Egypt. It was used for induction of type 2 diabetes mellitus by dissolving it in citrate buffer pH 4.5 and given as a single intraperitoneal injection[5]. Metformin hydro-chloride was purchased as tablets 500 mg from MINAPHARM Pharmaceuticals Company (10th of Ramadan City, Egypt). It was dissolved in distilled water and given by oral gavage.

Experimental design

Rats were categorized into the following three homogenous equal groups of eight rats each:

1- Control group (C): rats received normal pellet diet (NPD) daily. They were neither diabetic, nor MET treated [6].

2- Type 2 Diabetic non treated group (D): rats received high fat diet (HFD) daily for the whole period of experiment (12 weeks) excluding the two weeks of acclimatization, then after 2 weeks of HFD were injected with a single intraperitoneal (IP) dose of STZ (30 mg/kg) dissolved in citrate buffer (0.1 pH 4.0) to induce T2DM and then maintained on HFD till the end of experiment [7, 8].

3- Diabetic Metformin treated group (DM): rats with STZ induced T2DM, received daily Metformin (500 mg/kg body weight by gavage for the last 4 weeks of the experiment) and maintained on HFD daily till the end of the experiment [9].

The following tables shows the composition of both standard and HFD, standard NPD (12% of calories as fat) and HFD (58% fat, 25% protein, and 17% carbohydrate, as a percentage of total kcal).

Table (1): Composition of normal pellet diet (NPA) [10].

Ingredients	
proteins	**21.5 %**
Carbohydrates	**65 %**
Fat	**4 %**
Others (fibers, vitamins, and minerals)	**9.5 %**

Table (2): Composition of high fat diet (HFD) [11, 12].

Ingredients	**g/kg**
Normal Pellet diet	365
Clarified Butter	310
Casein	250
Cholesterol	10
Vitamins and Mineral mix	60
dl-Methionine	3
Sodium chloride	1
Yeast powder	1

Sample collection

After 4 weeks of treatment, all groups were fasted overnight and sacrificed by decapitation. Blood samples were withdrawn from the jugular vein, left to clot at room temperature, and then centrifuged at 3000rpm for 15 minutes in a cooling centrifuge. The serum layer was then collected into identified Eppendorf tubes and stored at -20°C until the time of assay [13].

The following parameters were assayed;

Glucose level (Direct Enzymatic Colorimetric Method using Glucose Kit Bio-diagnostic, Cairo, Egypt).

Insulin level (Immunoassay by Sandwich-ELISA principle using Rat Insulin ELISA kit, Elabscience-Biotechnology, Texas, USA).

HOMA-IR (Calculated from the following equation;

$$\frac{\text{fasting glucose (mg/dL)} \times \text{fasting insulin (μIU/ml)}}{405.}$$

MDA (Colorimetric Method using Lipid peroxide malondialdehyde (MDA) Kit (Bio-diagnostic, Cairo, Egypt).

TG (Enzymatic Colorimetric Method using Triglycerides Kit (Bio-systems, Barcelona, Spain).

LDL (Determined by the Friedewald equation as follows: LDL cholesterol (mg/dl) = total cholesterol − HDL cholesterol − [triglycerides/5]).

HDL (Enzymatic Colorimetric Method using High-density lipoprotein cholesterol Kit (Bio-diagnostic, Cairo, Egypt).

Urea level (Enzymatic Colorimetric Method using Urea Kit (Bio-diagnostic, Cairo, Egypt).

Statistical analysis:

The data were expressed as the means±Standard error of mean (S.E), comparisons between multiple groups were performed using one-way analysis of variance (one-way ANOVA), followed by the least significant difference (LSD) test. Results with $p < 0.05$ were considered statistically significant. Data were analyzed using IBM SPSS 28.0 statistical pack-age software (IBM; Armonk, NewYork, USA) [14].

Results

Effect of diabetes mellitus on glucose, insulin, HOMA-IR, TGs, LDL, HDL, Urea, and MDA.

The induction of T2DM using HFD and single low dose STZ resulted in impaired glucose tolerance with decreased insulin level and increased insulin resistance as evident by HOMA-IR. It also resulted in Hyperlipidemia in the form of increased levels of TGs and LDL and decreased level of HDL. Regarding Urea and MDA levels they were found to be increased in the sera of the diabetic rats.

Effect of metformin on serum glucose, insulin, and HOMA-IR

Administration of metformin to diabetic rats significantly lowered the serum glucose level. In addition, it showed a significant increase in the serum insulin level and a significant decrease in HOMA-IR when compared to that of the diabetic non treated group.

Effect of metformin on Urea

Metformin administration to diabetic rats resulted in decrease in the serum level of urea which was significant when compared to diabetic non treated group, but still significantly higher when compared to control group.

Effect of metformin on TGs, LDL, and HDL

Administration of metformin to diabetic rats resulted in significant decrease in the serum levels of TGs and LDL with significantly increased serum level of HDL as compared to the diabetic non treated group, however TGs and LDL were still significantly higher and HDL level was significantly lower than the corresponding levels of the control group.

Effect of metformin on MDA

Metformin administration also resulted in a significant lower level of serum MDA when compared to the diabetic non treated group.

Table (3): Effects of Metformin on glucose, insulin, HOMA-IR, TGs, LDL, HDL, Urea, and MDA in the diabetic rats 'serum.

Parameters		Groups	
	C	D	DM
Glucose (mg/dl)	101.2 ±1.9	306.5[a] ±15.7	189.1[b] ±3.1
Insulin (µIU/ml)	7.33±0.15	4.87[a]±0.29	6.17[b]±0.14
HOMA-IR	1.83±0.03	3.63[a]±0.08	2.87[b]±0.03
Urea (mg/dl)	21.3±1	47.5[a]±1.34	26.8[a,b]±0.5
TGs (mg/dl)	94.98±2.3	195.2[a]±1.3	124.2[a,b]±1.6
LDL (mg/dl)	23.26±1.18	74.43[a]±2.1	38.5[a,b]±2.8
HDL (mg/dl)	47.97±1.6	30.28[a]±0.3	40.94[a,b]±0.8
MDA (nmol/ml)	5.05±0.49	10.6[a]±0.32	6.99[b]±0.44

Data are expressed as M±SE. The mean difference between groups was considered statistically significant when P value < 0.05. C: Control group. D: Diabetic non treated group. DM: Diabetic + Metformin treated group.

- [a] significant with the corresponding mean of the control group.
- [b] significant with the corresponding mean of the diabetic non treated group.

Discussion

Diabetes mellitus is a group of metabolic disorders characterized by chronic hyperglycemia with disturbances in carbohydrates, fat, and protein metabolism resulting from defects in insulin secretion, insulin action, or both. The chronic hyperglycemia is associated with dysfunction and failure of various organs. Several pathogenic processes are involved in the development of diabetes ranging from autoimmune destruction of the beta cells of the pancreas with subsequent insulin deficiency to abnormalities that result in resistance to insulin action [15].

The induction of T2DM was done using HFD for a period of two weeks to induce obesity with its subsequent insulin resistance, hyperinsulinemia, and glucose intolerance. This dietary regimen was followed by low dose of a freshly prepared STZ solution dissolved in cold citrate buffer (pH 4.5) at a dose of 30 mg/kg body weight given IP in a single dose to the overnight fasted rats. This STZ injection causes moderate destruction in the pancreatic islets of Langerhans resulting in imbalance between insulin resistance and hyperinsulinemia and causes hyperglycemia. So, this rat model of HFD+STZ was progressed from insulin resis-tance to hypoinsulinemia and hyperglycemia [16].

Hyperlipidemia is a major character of T2DM and one of the most common features of STZ-induced hyperglycemia in experimental rats. It is characterized by high levels of LDL and TG with low level of HDL. Hyperlipidemia is attributed to excess mobilization of fat from adipose tissue due to underutilization of blood glucose [17].

Metformin (MET) is widely used as a therapy for T2DM because of its desirable effects, high safety, and reasonable cost. It exerts its antidiabetic effect by decreasing the hepatic gluconeogenesis and boosting insulin sensitivity to control the abnormally high blood glucose levels and decreasing associated diabetic complications as well as it possesses marked anti-inflammatory, anti-oxidative, anti-apoptotic, and anti-cancer activities [18, 19].

The results of the present study revealed that administration of MET to diabetic rats was able to significantly decrease the blood glucose level and HOMA-IR as compared to diabetic non treated rats [20] and significantly able to increase serum insulin level probably by enhancing expression and function of GLUT-4 in cells and by stimulating Glucagon like peptide 1 (GLP-1) release, both effects enhance the secretion of insulin from pancreatic bets cells [21].

MET administration to diabetic rats also resulted in significant decrease in the serum level of urea signifying improvement in renal function. It also induced a significant lipid-lowering effect reflected by significant decrease in TGs and LDL serum levels and a significant increase in HDL serum level which is compatible with [22, 23]. MET affects the lipoprotein synthesis in the intestine of diabetic rats by reduction of mRNA expression of genes responsible to the intestinal lipid homeostasis [24].

Chronic hyperglycemia associated with T2DM is a main cause in depletion of the intracellular anti-oxidant enzymes system with consequent development of oxidative stress resulting in massive intracellular free radicals accumulation[25]. MET treatment resulted in an obvious increase in total anti-oxidant enzymes activities with decrease in lipid peroxidation as evident by low level of MDA indicating the improved scavenging activity of ROS, this could be a result of MET ability to activate AMPK and increase the expression of its target proteins[26].

Conclusion

The present study concluded that the experimental model of T2DM in rats is characterized by hyperglycemia, and increased insulin resistance associated with increased oxidative stress markers, disturbed atherogenic lipid profile and renal function markers. A model that resembles T2DM in humans. Metformin treatment in a dose of 500 mg/kg/day for four weeks could partially reverse the diabetic changes but not to the control healthy level. So, MET is not absolutely effective and combinations with other anti-diabetic drugs must be tried.

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