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Research Article

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A Comparative Study on the Effects of Incretin and Metformin on Lipid Profile and Oxidative Stress in Diabetic Rats

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ABSTRACT

Type 2 diabetes is a prevalent and growing problem worldwide. This study aimed to evaluate the effect of metformin and sitagliptin on lipid profile and oxidative stress in streptozotocin (STZ) induced diabetic albino Wistar rats. Twenty four neonatal rats (approximately 9±2 g), were randomly divided in to 4 groups: The control (n=6), untreated diabetic (n=6), diabetic treated by metformin (n=6) and treated by sitagliptin (n=6). The diabetes was induced in the subjects by intraperitoneal injecting of STZ with a dose of 90 mg/kg body weight. Then, the rats were kept 8 weeks under the same conditions then enrolled in the study. The group 3 and 4 was respectively gavaged by metformin (150 mg/kg/day) and sitagliptin (100 mg/kg/day) for one month. At the end, blood samples were collected from each rat. Moreover, the serum levels of triglyceride (TG), total cholesterol (TC), HDL-C, LDL-C and Malondialdehyde (MDA) were assessed.In diabetic rats, metformin therapy reduced serum concentration of TG (-82.2±6 mg.dl), TC (-55.8±1 mg.dl), LDL-C (-68.46 ±7 mg.dl), MDA (-1.7±0.18 µm.L) and increased serum concentration of HDL-C (+29.1 ±0.1 mg.dl) (significant at P ≤0.05). Also in diabetic rats, sitagliptin therapy reduced serum concentration of TG (-75.9±5 mg.dl), TC (-50.1±9 mg.dl), LDL-C (-62.12 ±6 mg.dl), MDA (-1.5 ±0.07 µm.L) and increased serum concentration of HDL-C (+27.2 ±0.7 mg.dl) (significant at P ≤0.05). As the results show, about the parameters studied in this experiment, there was no significant difference between 2 groups that treated with metformin and treated with sitagliptin in the end. In other words, sitagliptin is effective on improving the lipid profile and oxidative stress like metformin. Findings of this study showed that sitagliptin is an efficient pharmaceutical composition with beneficial antidiabetic effects. However, since the effect of this drug is dependent on secretion of endogenous GLP-1, it should be used only for patients with some extent of active β-cells.

Keywords: Diabetes Mellitus, Incretin, Metformin, Sitagliptin, Lipid profile, Oxidative Stress, Malondialdehyde (MDA)

INTRODUCTION

The prevalence of type 2 diabetes and the associated morbidity and mortality are increasing (1, 2). Therefore, there is special concern with strategies to curtail the risks or prevent the development of type 2 diabetes. Although insulin resistance as a secondary consequence of diabetes leads to lifestyle changes in the patient followed by an increase in the prevalence of diabetes in the society, the insulin secretion increases in most resistant individuals who remain non-diabetics (3, 4). Conversely, insulin resistance in genetically vulnerable individuals aggravates type 2 diabetes, reduces the cellular activity β islets and ultimately, results in hyperglycemia(4-6). The type 2 diabetes is characterized by a gradual deterioration of β-cell function looking for insulin resistance. Moreover, it is

accompanied by escalation of blood glucose control, which in turn leads to hyperglycemia related to the complications of diabetes, including diabetic retinopathy, nephropathy, neuropathy, dyslipidemia and cardiovascular disease (CVDs)(7). Diabetes mellitus (DM) involves a group of metabolic disorders with various etiologies which lead to increased blood sugar and impaired glucose, fat and protein metabolism (8). It seems that the metabolic disorder of the sugar and insulin- resistance play a pivotal role in the incidence of dyslipidemia in people with type 2 diabetes (4). On the other hand, one of the outcomes of diabetes is oxidative stress that is caused by the effect of hyperglycemia (8).

Recent studies indicate that oxidation agents in diabetes result in many complications (8).The main reason for the emergence of these symptoms is the outset of Millard chain chemical reactions after the glycation of proteins, which later leads to formation of Schiff base, Amadori products and eventually advanced glycation end products(AGEs)(9, 10). The AGEs are non-application proteins that amass together and can alter the structure and function of other proteins. They can also affect the neural pathways where messages are transferred (9-11).The baby albino Wistar rats with streptozotocin-induced diabetes are particularly useful for testing the insignificance of β-cell function in the development of type 2 diabetes (NIDDM). In this model, diabetes is induced by injection of STZ at different doses (80-100 mg/kg) at various age groups (0, 2 or 5 days of birth) leading to different degrees of β-cell destruction(12).Previous studies have shown that lower glycation of proteins and higher stability can specifically reduce the risk of diabetes complications (13, 14).As a first- line therapy Proper diet and exercise promote weight loss and improve blood glucose control in individuals who have recently been diagnosed with diabetes(4). However, most patientswill need to take oral antidiabetic drugs (OADs) so as to control blood glucose and many more will ultimately require insulin therapy. Although these treatments are effective in lowering HbA1C, they often lead to weight gain in patients. For example, sulfonylureas, thiazolidinediones and insulin lead to weight gain by 2 kg per 1% reduction of HbA1C(15, 16).By comparing the performance of metformin (golden drug that is already widely used to treat type 2 diabetes) and incretin (a new generation of blood glucose lowering drugs) and investigating beneficial effects on lipid profile and oxidative stress, we can more accurately understand their mechanism and then finally apply new detection and treatment methods for type 2 diabetes patients using this drug family.Of the available therapies, metformin is the most commonly used oral antihyperglycemic agent (OHA), both as monotherapy and in combination with other agents such as sulfonylureas or thiazolidinediones(17, 18). Metformin reduces glucose production in the liver and improves insulin resistance so as to curtail blood glucose in diabetic patients (19). In addition, metformin has been reported to increase by 1.5 to 2-fold the concentrations of biologically active glucagon like peptide-1(GLP-1) in non-diabetic individuals suffering from obsess under the effect of oral glucose (20). The effect on the concentration of GLP-1 is not a consequence of inhibiting enzyme activity of dipeptidyl peptidase-4(DPP-4)(21, 22). Metformin, unlike other standard oral anti diabetes drugs (OADs), is often associated with slight weight loss (23).In response to food intake, the digestive tract secretes a great deal of gastrointestinal hormones, among which glucose-dependent insulinotropic peptide (GIP) and GLP-1 are important in regulation of blood glucose. Generally, GLP-1 and GIP are known as incretin hormones. Both stimulate the insulin secretion from pancreas β-cells in response to intake of oral glucose. Furthermore, GLP-1 can inhibit the secretion of glucagon after meals. Under normal physical conditions, the activity of these hormonesleads to limiting the rise in blood glucose levels after eating. In their absence, however, the pancreatic response to glucose concentration decreases. This is technically called incretin effect first demonstrated in 1960s. These hormones are responsible for the secretion of 50 to 70 percent of insulin release after consumption of glucose. Moreover, they are able to function so after food intake and before the blood glucose level increase. It has been observed that oral administration of glucose increases insulin secretion more intensely than when it is administered intravenously, thus, these findings identify the function of incretin to a certain extent(24, 25).

The incretin-based therapies include the use of GLP-1 receptor agonists and DPP-4 inhibitors, which have been recently prescribed for controlling type 2 diabetes. In contrast to many traditional treatments, they never lead to weight gain in patients (26). Sitagliptin, vildagliptin, Sazagliptin and linagliptin from the DPP-4 inhibitors are relatively new hypoglycemic drugs lately approved by the US Food and Drug Administration or the European Medical Organization. Nevertheless, other drugs from the same family are awaiting approval or in the development stage (27). Sitagliptin is an oral medication and highly selective DPP-4 inhibitor demonstrating a new approach for the treatment of patients with type 2 diabetes (28). The DPP-4 inhibitors can prevent the enzymatic analysis of GLP-1 and GIP (two incretin hormone involved in glucose homeostasis) (29). On the basis of studies done in the past, following the oral glucose tolerance test, Sitagliptin resulted in a two-fold increase in concentrations of biologically active GLP-1 and GIP as well as higher insulin secretion and lower glucagon secretion compared to placebo in patients with type 2 diabetes (30). These changes would ultimately lead to a significant decrease in postprandial

blood glucose levels (31).This study was designed in line with several recent research on diabetes covering the effect of aqueous extract of saffron (Crocus Sativus) on serum biochemical parameters in rats with STZ-induced diabetes (32), overview of mechanisms in plant nutrients during the treatment of diabetes (33),effect of Purslane extract on antioxidant balance in women with type 2 diabetes (34), changes in levels of AGEs and β2-microglobulin and imbalance of trace elements in type 2 diabetes37,this study designed.

MATERIALS AND METHODS

Providing laboratory animals and diabetic injection

The baby albino Wistar male rats (purchased from an animal house of Jundishapur University, Ahvaz) with an average age 2 to 5 days divided randomly into two healthy and patient groups. The latter was intraperitoneally (IP) injected STZ (90 mg/kg body weight). Healthy group as a control only received physiological serum injection (12).

Classification of animals

After 48 hours, the rats with blood glucose levels greater than 250 mg/dl were considered diabetic and used in the experiment (35). After confirmation of diabetes and spent 8 weeks, the rates were divided into 4 groups of six : 1) Normal rats considered as a control after serum injection without STZ , 2) Diabetic rats without treatment , 3) Diabetic rats treated with metformin (150 mg/kg body weight) (35), and 4) Diabetic rats treated with sitagliptin (100 mg/kg body weight) (25, 36). This study was carried out for a month.

Blood and Serum Samples

After one month, rats were sacrificed and blood samples were taken from the Aortic input. These blood samples were kept at room temperature for 60 minutes and serum was isolated away through centrifuge at 3000 rpm for 15m at 25˚c. Serum falcons were kept at-20˚c until the analysis of biochemical parameters.

The experiment protocol was confirmed by the Council of animal ethics committee with instructions regarding the use and treatment of laboratory animals prepared by Ahvaz Jundishapur University of Medical Sciences.

Biochemical Analysis

The concentration of the total cholesterol (TC) (λ=520nm) and triglyceride (TG) (λ=505nm) were measured by commercial kits (Bionik, Tehran, Iran), automatic analyzer (Abbott, model Alcyon 300, USA) and spectrophotometer device based on enzymatic colorimetric methods.

The high density lipoprotein cholesterol (HDL-C) of serum samples was purified by quantitative detection kit (HDL-C precipitant. Pars Azmone, Tehran, Iran) according to precipitant all lipoproteins (chylomicron, VLDL and LDL-C) in response to reaction with phosphotungistic and magnesium ions. Finally, concentration of HDL-C was measured by enzymatic colorimetric method using spectrophotometer at 546nm.The concentration of low density lipoprotein cholesterol (LDL-C) of serum samples was calculated using computational method of Fried Wald equation.

LDL – C (mg/dL) = Total cholesterol – (VLDL + HDL- C)

To study the effect of drugs on oxidative stress, lipid peroxidation was measured by determination of serum concentration of malondialdehyde (MDA) by chemical method with commercial kit (TBARS) according to the kit instructions. The reaction of MDA with thiobarbituric acid (TBA) (PH≥7, T=90˚c) composed the pink complex which its absorbance was measured by spectrophotometer at 532nm and adapted with standard curve based on dilutions prepared by tetra etoxy propane. All the results were presented as mean ± SD. differences between mean values for each parameter in different groups were specified through one-way ANOVA at significance level of P≤0.05 using SPSS 16.0.

RESULTS

Figures 1 to 4 show the variations in serum lipid profile of rats at the end of the experimental period for one month. According to Figure 1, 2 and 3, respectively levels of TG, TC, and LDL-C in diabetic group (D) increased as compared to the control group (H) at significance level at P≤0.05. Finally, levels of those parameters decreased after

lapse of one-month period in diabetic rats treated with metformin (D+M) and treated with Sitagliptin (D+I) at significance level of P≤0.05.

According to Figure 4, the serum concentration of HDL-C in diabetic group (D) at significance level of P≤0.05 was lower than this compared to the normal group (H). At the end of one-month experimental period, HDL-C in diabetic rats treated with metformin (D+M) and sitagliptin (D+I) increased significantly at P≤0.05. According to data obtained, metformin and sitagliptin were effective in improving HDL-C levels and there were no significant differences between them.

Figures 1 to 4. Illustrate the variation in concentrations of serum lipid profile in rats at the end of one-month treatment. (1)TG, (2)TC, (3)LDL-C and (4)HDL-C. In 4 groups: healthy control (H), diabetic without treatment (D), diabetic treated by metformin (D+M) and diabetic treated by sitagliptin (D+I).

Fig 5 shows that the concentration of MDA in diabetic rats was significantly (P≤0.05) higher than that in the control group. This parameter in diabetic rats receving metformin (D+M) and sitagliptin (D+I) was reduced significantly (P≤0.05). But, there was no statistically significant association between 2 diabetic groups treated with metformin (D+M) and sitagliptin (D+I) at end of the experiment.

According to Figure 5, serum levels of MDA in the diabetic group (D) at significance level of P≤0.05 were higher than in the control group (H). This biochemical parameter decreased in diabetic groups treated with metformin (D+M) and Sitagliptin (D+I) at significance level of P≤0.05.

DISCUSSION

In diabetic rats, metformin therapy reduced serum concentration of TG (-82.2 ± 6 mg.dl), TC (-55.8 ± 1 mg.dl), LDL-C (-68.46 ± 7 mg.dl), MDA (-1.7 ± 0.18 µm.L) and increased serum concentration of HDL-C (+29.1 ± 0.1 mg.dl) (significant at P ≤ 0.05). Also in diabetic rats, sitagliptin therapy reduced serum concentration of TG (-75.9± 5 mg.dl), TC (-50.1 ± 9 mg.dl), LDL-C (-62.12 ± 6 mg.dl), MDA (-1.5 ± 0.07 µm.L) and increased serum concentration of HDL-C (+27.2 ± 0.7 mg.dl) (significant at P ≤ 0.05).Oral administration of metformin (150 mg/kg body weight) for one month improved the lipid profile and oxidative stress in STZ-induced diabetic rats. This indicates that metformin has anti-hyperglycemic and anti-lipidemic effects. According to researches carried, metformin improves lipid profile through activation of adenosine mono phosphate protein kinase (AMPK) that is effective in insulin signaling, energy balance of the whole body and metabolism of lipids (37). Activated AMPK inhibits acetyl-COA carboxylase (ACC) through phosphorylation of Ser-79 resulting in reduction of malonyl-COA level and activation of CTP-1 enzyme and thus inhibits lipogenesis (37).In addition, metformin provides the possibility of oxidation of fatty acids through activates lipoprotein lipase (LPL) to break down VLDL in to free fatty acids (FFA)(37).Metformin reduces level of cholesterol in patients with T2DM. This means that activated AMPK reduces synthesis of cholesterol through phosphorylation and inhibition of HMG-COA reductase (cytosolic enzyme of cholesterol biosynthesis). As well as, activated AMPK reduces biosynthesis of triacylglycerol (TAG) in liver and then reduces concentration of TG in diabetics (37). Oral administration of metformin prevents to increase the MDA level (as a lipid proxidation index). In other words, the increment of MDA level in serum of vistar albino rats (induced by STZ) shows that oxidative stress and reactive oxygen species (ROS) are effective in pathogenesis of type 2 diabetes (38). Sitagliptin with 100 mg/kg body weight dosage for one month improved the lipid profile and oxidative stress in STZ-induced diabetic rats. According to studies, sitagliptin inhibits DPP-4 enzyme and then prevents premature degradation of endogenous GLP-1.

GLP-1 causes exocytosis of insulin-containing granules through binding to its receptor on surface of β-cells, activation of protein kinase-A (PKA), increment of cAMP level and finally increment of intra cellular concentration of Ca^{2+}ions (39-41). On the other side, stimulation of β-cells receptors activates different pathways of tyrosine kinase cascade, increases pdx-1 gene expression and finally leads to stimulation of mitosis, neogenesis and inhibition of apoptosis (39, 40).As well as, GLP-1 causes reduction of glucagon secretion through binding to receptors of α-cells (39, 40).GLP-1 induces satiety signal and reduces food intake through stimulation of pyloric sphincter receptors and reduction of gastric emptying rate (39).In muscle, GLP-1 causes increment of glycogen synthesis after the meal through secondary messenger like IPG, IP3K/PKB and MAPkinase pathways (42, 43).Although GLP-1 synthesis happens in the brain, but only where the blood-brain barrier (BBB) allows, can bind to its receptor and induce satiety signal (44, 45).Given that the most important property of GLP-1 to be stimulate

insulin secretion in clinical trials, thus all of anti-diabetic effects of sitagliptin related to mechanism of insulin in body like the inhibition effect of insulin on hormone-sensitive lipase enzyme in adipose tissue which leads to inhibition of the degradation of TG into FFA, thereby inhibiting cholesterol synthesis or the stimulation effect of insulin on ATP-citrate lyase, acetyl-COA carboxylase (ACC), fatty acid synthase (FAS) and glucose 6-phosphate dehydrogenase (G6PDH) which lead to increase adipose tissue lipogenesis (46).The increment of insulin secretion by oral administration of sitagliptin leads to increase intake of glucose by peripheral tissues and as a result reduce production of advanced glycated end products (AGEs). The low level of AGEs causes reduction concentration of free radicals, and proxidation of unsaturated fatty acids in membranes and lipoproteins and finally reduces production of reactive dialdehyde compounds such as MDA (46).As results show, about parameters studied in this experiment, there was no significant difference between 2 groups that treated with metformin and treated with sitagliptin in the end. In other words, sitagliptin is effective on improving the lipid profile and oxidative stress like metformin. Based on findings of this study and its relation to previous studies that have been done, it can be concluded that sitagliptin is suitable as a pharmaceutical composition and has beneficial antidiabetic effects. But since the effect of this drug is dependent on secretion of endogenous GLP-1, can be used only for patients with somewhat active β-cells.

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