Concomitant use of Dopamine agonist Bromocriptine with Glimepride Improves Insulin Sensitivity in Type II Diabetic Patients

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Abstract: *Type -2 diabetes mellitus (T2DM) is a complex syndrome originated by a multifactorial pathogenesis. Restoration of a normal glycaemia is very difficult and requires a multiple medication with different mechanismsof action that can be used in combination to produce an additive effect. Bromocriptine (BC) is a sympatholytic D2 dopamine agonist which can be used as an anti-diabetic agent with a novel mechanism of action in combination with a currently used drug glimepiride.*
Objective*: The aim of this work is to study theeffect ofaddition of BC toglimepiride inuncontrolled type 2 diabetic patients treated with glimepiride.*
Design*: we collect the type 2 diabetic patients which treated with glimepiride and not achieved a glycemic control and compare their results with other glimepride-treated group with combination with 1.25 mg BC once daily. Three groups divided randomly: the first group take glimepiride 3mg once daily, the second group takes glimepiride 3 mg once daily plus 1.25 mg of BC once daily, the third group is the control group. A control group comprising healthy, normal glycemic individuals was used for comparison.The effect of BC on glucose homeostasis parameters (e.g. blood glucose level, serum insulin, insulin resistance using the homeostasis model assessment), lipid profile, and dopamine and adiponectin levels are investigated.*
Keywords: *Adiponectin, Bromocriptine,Dopamine, Glimepride, Insulin resistance, T2DM*

I. Introduction

Type 2 diabetes mellitus (T2DM) is a chronic metabolic disease characterized by impairedβ-cell function, increase hepatic glucose production and reabsorptionandincrease glucagon productionand on the other hand elevation of insulin resistance **(Sung & Kim, 2011)**.Obesity, and especially visceral adiposity, escalates the development of insulin resistance in T2DM. Excess adipose tissue contributes to a chronic increase in circulating fatty acids reducing the usage of glucose as a source of cellular energy. Excess fatty acids also result in increasing deposition of fat in muscle and liver, and increasing metabolites such as diacylglycerol and ceramide which activate isoforms of protein kinase C that impede cellular insulin signaling. Chronically raised lipid levels also impair islet beta cell function, acting in conjunction with insulin resistance to aggravate hyperglycemia **(Marrero et al., 2014)**.

In adipose tissue, increased energy storage leads to not only an accumulation of lipids but also inflammation, including the infiltration and activation of immune cells. This interaction between adipocytes and immune cells results in the altered secretion of adipokines, which significantly affects the metabolic state of other tissues including the liver, skeletal muscle, brain, and vascular system. Adiponectin is a representative adipokine that has been shown to be a biomarker of T2DM and CVD and is also involved in the pathogenesis of these disorders **(Hung et al., 2008)**.

Glycemic control is a fundamental part of the management of T2DM. Antidiabetic drugs have a different mechanisms of action such as stimulate insulin secretion, reduce gluconeogenesis, improve insulin resistance and peripheral glucose uptake,increase insulin sensitivity, decrease intestinal glucose absorption, glucose reabsorption and improve physiological incretin. Achievement of glycemic control is difficult and requires multi-antidiabetic medication in combination with multiple daily administration to produce an additive effect with subsequent various effects so the development of antidiabetic agents with novel mechanism of action is highly recommended **(Khalil et al., 2016)**.

Glimepiride is a second generation sulphonylurea which enhances insulin secretion through binding to the sulphonylurea receptors (SUR1) on pancreatic β-cells and thereby causes glucose-independent closure of the ATP-sensitive K^+ channels.Glimepiride could also exert extrapancreatic effects such as improving peripheral glucose uptake, insulin sensitizer effect, and suppression of endogenous glucose production **(Engbersenet al., 2012)**.After a time of treatment patients become glycemic uncontrolled and increase the dose will leads to that the β cell become so exhausted and the choose of another drug to increase sensitivity of receptor to insulin is more logic.In addition to low durability, hypoglycemic episodes lead to fear of further episodes, which may lead

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to patients eating more to avoid their blood glucose becoming too low, resulting in an association between hypoglycemia, fear of hypoglycemia and weight gain **(Marrett et al., 2009; Alvarsson et al., 2010)**.

In addition to glucose stimulation, however, multiple G-protein coupled receptor (GPCR) ligands also play a large role in the modulation of insulin release. GPCRs are common therapeutic targets and understanding of the mechanisms by which these ligands, in general, and dopamine, in particular, modulate insulin release is of increasing importance.The role of dopamine as a paracrine/autocrine regulator of insulin secretion from the β-cell in the pancreatic islethas been complicated because both stimulation and inhibition of insulin secretion have been reported **(Winzell&Ahre´n, 2007)**. Although the role of dopamine in endocrine cells has been controversial, there is now strong evidence lead to a putative physiologic role for dopamine in modulating insulin secretion by the existence of a dopaminergic negative feedback loop acting on the endocrine pancreas **(Ustione et al., 2013)**.

Otherwise, dopamine release at hypothalamic suprachiasmatic nuclei (SCN) has been implicated in the regulation of peripheral insulin sensitivity and glucose and lipid metabolism. Other studies have identified the ventromedial hypothalamus (VMH) as an additional target for such metabolic influences of hypothalamic dopaminergic activityin modulating autonomic nervous system function, hormonal secretion, peripheral glucose/lipid metabolism, and feeding behavior **(Moe et al., 2014)**.A large body of evidence implicates endogenous dopaminergic and serotonergic rhythms in SCN and VMH in the transition from the insulin-sensitive to insulin-resistant state. Conversely, dopamine levels are low during the insulin-resistant state and increase to normal following return of the insulin-sensitive state. Further, selective destruction of dopaminergic neurons in the SCN causes severe insulin resistance **(Shivaprasad&Kalra, 2011)**.

Bromocriptine (BC) is a sympatholytic D2-dopamine agonist that can reduce sympathetic activity and levels of circulating norepinephrine and thus contribute to the management of T2DM. No evidence that it has a specific receptor that mediate its action on glucose and lipid metabolism neither augment insulin secretion nor enhance insulin sensitivity in peripheral tissues. Rather, its effects are mediated via resetting of dopaminergic and sympathetic tone within the CNS resulting in a reduction in post meal and fasting plasma glucose levels **(Via et al., 2010; Ustione&Piston., 2012)**.

The aim of this study was to address the hypothesis that BCaddition to poorly controlled T2DM patients treated with sulphonyl-urea glimepiride can be ameliorated.

II. Patients and Methods

2.1 Patients

The study included a total of 40 obese insulin-resistant T2DM uncontrolled patients which were randomly enrolled in the study and planned to receive glimepride-based regimen. The patients were recruited from the Diabetes Clinic, Internal Medicine Department, Tanta University Hospitals, from June 2015 to September 2015. Patients with history of type 1 diabetes mellitus, kidney dysfunction, or impaired liver functions were excluded. Also, 15 healthy volunteers with matched age and sex served as control group. All subjects were informed about the study and gave an informed consent. The study was carried out according to the ethical guidelines approved by the Ethical Committee at Faculty of Pharmacy, Tanta University.

The study participants were under treatment with Amaryl® (glimepride3 mg/day(Sanovi Aventus. Company, Egypt) forone to three years. Blood samples were collected from them at the start of study, then, glimepride-treated patients were supplemented with oral prolactin® (Bromocriptin2.5 mg/day(Amoun Company, Egypt)which was approved to be taken in a range 1.6-4.8 mg/day through 2 hour of wakening as adjuvant therapy for 2months, after which second blood samples were collected. The patients were followed closely to ensure that they stacked to the treatment.

Detailed history of patients was recorded. According to **American Diabetes Association (2014)**, uncontrolled Diabetic status was categorized as 'T2DM', diagnosed when the current treatment regimen does not keep the blood sugar level within acceptable levels. The weight and height of patients were measured and body mass index (BMI) was calculated by dividing the patient weight (kg) by patient height (m^2). The characteristics of the study participants are illustrated in **Table (1)**.The mean age of glimepride-treated patients was 54.3 years. The majority of patientsenrolled in the study were had BMI 30 kg/m^2 or greater.The diagnosis was confirmed by Fasting Blood Glucose (FBG) > 126 mg/dL**(Expert committee on the diagnosis and classification of diabetes mellitus report, 2003)**.

Blood was drawn by venous arm punctures from patients after overnight fast.five mL of blood was added immediately to plain tube and the serum was separated by centrifugation at 4000 rpm for 15 min using 5804 centrifuge (Eppedorf Company, German). Serum was divided into 2 portions. First portion of blood allowed for immediate analysis of glucose, insulin and lipid profile. The second portion was stored frozen at -70 °C till the time of determination of adiponectine and dopamine levels.

2.2. Determination of Glucose, Insulin and hemostasis model of insulin resistance

Serum glucose level was estimated by using an Accu-Check Active glucometer. While, the level of serum insulin was assayed using anenzyme linked immunosorbent assay (ELISA) according to **Andersen et al, 1993** using kits from BioSource Europe S.A.The insulin resistance index (IRI) was evaluated by homeostasis model assessment estimate of insulin resistance (HOMA-IR) as follows:

HOMA-IR= Insulin x Glucose/405 (**Matthews et al, 1985**).

2.3. Measurements of lipid profile

Enzymatic colorimetric determination of total cholesterol was according to **Allain and coworkers, (1974)** using kits obtained from Greiner Diagnostic Company, German. Serum level of triglycerides was determined according to **Bucolo and David**, **(1973)**using kits obtained from Elitech Diagnostics Company, France. High density lipoprotein cholesterol (HDL-C) was determined using kits obtained from Randox Laboratories Ltd Company, England. Low density lipoprotein cholesterol (LDL-C) was calculated according to Friedewaldformula(**Friedewaldet al., 1972).**

2.4 Measurement of adiponectine and dopamine

Quantitative measurement of serum adiponectine and dopamine was carried out by enzyme-linked immunosorbent assay (ELISA) using kits obtained from Assaypro® Human adiponectin and SUN RED BIO® Human DA (dopamine), respectively. The intensity of the color was measured at 450 nm using microplate reader (TECAN Austria GmbH 5082 Grodig, Austria).

2.5. Data Analysis

Data are presented as mean ± standard deviation (SD) and were analyzed by Microsoft software (EXCEL 2000) and Statistical Package for Social Science (SPSS) version 17. The experimental data were analyzed for significant differences by paired t-test. The level of significance was set at $P<0.05$.

III. Results

At the beginning of the study, all diabetic patients showed significantly ($P<0.05$) higher levels of FBG (hyperglycemia) compared to the control patients (**Table 1**). By the end of the treatment period (after 8 weeks), all treated patients in both glimepride and glimepride BC group showed significant ($P<0.05$) reduction in FBG when compared to the before treatment in spite that it still significantly ($P<0.05$) higher compared to normal control . Although glimperidetreated group showed FBG decreased by 21.9% at the end of the study, in glimeprideBC group FBG decreased by 32.3%. Also, at the start of the study, all diabetic patients showed higher insulin levels compared to the control normal group (**Table 1**). By the end of the treatment period, diabetic groups treated with either glimepiride at dose of 3 mg/dayor glimeprideconcomittent with BC showed significant ($P<0.05$) lower levels than the untreated diabetic patients.

The insulin resistance index calculated by the HOMA model (HOMA-IR) using the level of fasting insulin (μIU/mL) and glucose level (mg/dL) indicated that all diabetic groups started the experiment with significantly ($P<0.05$) higher HOMA-IR values compared to the control group. At the end of the treatment period, all of the treated patients showed a significant decline in the insulin resistance index compared to the pretreatedpatients with the least value observed in patients treated with bromocriptine(**Table1**). Although,glimperidetreated group showed HOMA IR decreased by 56% at the end of the study, in glimeprideBC group HOMA IR decreased by 70%.

Table 1: Glucose homeostasis parameters of different studied participants

Group		Glimperide group		Glimepride+Bromocriptine group		Control group
	Number		15		15	10
	Sex M/F		4/11		4/11	4/11
Age(years)	Range		40-55		42-60	45-53
BMI(Kg/m2)	Range		28-32		28-32	25-29
FBG mg/dL	Range	Before	After	Before	After	70 - 118
		150 - 292	110 - 222	140 - 270	90- 170	
	Mean	186.8 [a] ± 11.1	145.93 [a,b] ± 8.48	188.5 [a] ± 11.2	127.67 [a,b] ± 5.63	100.47 ± 3.74
Fasting insulin(μIU)	Range	28.4 - 33.3	15.5 - 19.3	28.8 - 34.0	12.3 - 19.0	15.5 - 19.1
	Mean	30.41 [a] ± 0.40	16.88 [b] ± 0.35	30.89 [a] ± 0.41	16.07 [a,b] ± 0.49	17.37 ± 0.23
HOMA IR	Range	10.50 - 23.0	4.64 - 9.37	7.63 - 22.31	3.43 - 6.97	2.18 - 4.24
	Mean	14.00 [a] ± 0.83	6.04 [a,b] ± 0.32	17.90 [a] ± 1.18	5.35 [a,b] ± 0.23	3.65 ± 0.19

a: Significant versus contro normal, b: Significant versus glimepride treated group, FBG: Fasting blood glucose, BMI: Body mass index, HOMA-IR: Homeostasis model assessment of insulin resistance

glimepride-treated patients showed significant increase (P<0.05) in serum T-C,TGs, VLDL and LDL-C levels compared to the control patients. The level of T-C,TGs, VLDL and LDL-C were150±10.7, 125.3±7.3, 25±3.5, 85±4.9mg/dL respectively in control group, increased to 200.4±40.3, 183.7±65.8, 36.7±13.2 and 123.7±31.0 mg/dLrespectivelyin glimepride-treated group. Otherwise, HDL-C wasnon-significantly changed in glimepride-treated patients when compared to control group(**Figure 1**).

Supplementation of glimepride-treated patients with BC produced a significant decrease in TGs and VLDL-C levels (P<0.05). The levels of lipid parameters were 125.3 ±14.9 and 26.7±3.0 mg/dL for TGs and VLDL-C, respectively, in glimepride-treated patients-after BC supplementation. On the other hand, All lipid parameters in glimepride+BC group were non-significantly changed when compared to control groupexcept for LDL-C lipoprotein which still significantly increased to 107.1±19mg/dL.
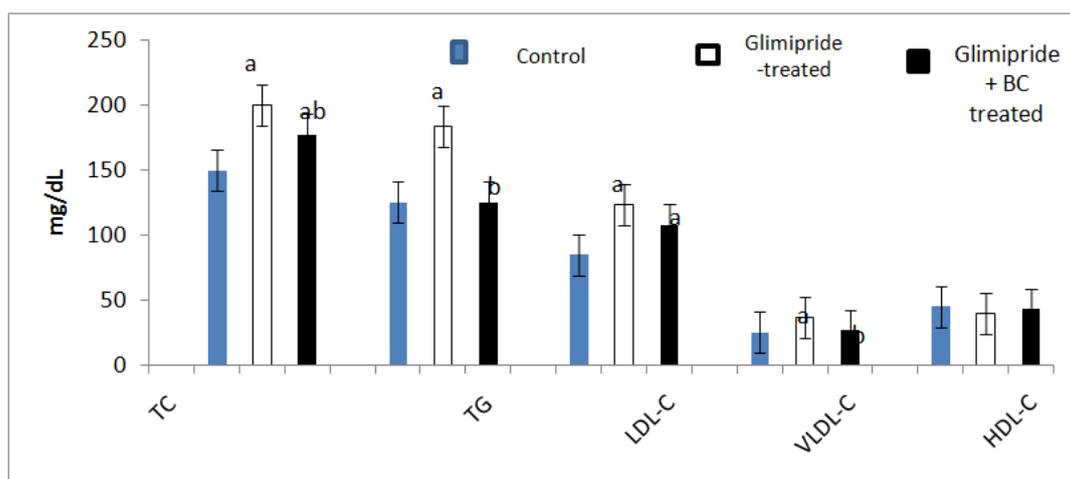

Figure (1): Levels of Total cholesterol (TC), Triglycerides (TG), Low density lipoprotein cholesterol (LDL-C), Very low density lipoprotein cholesterol (VLDL-C) and High density lipoprotein cholesterol (HDL-C) in the studied T2DM patients at the end of the study. Data are presented as mean±SE.

a: Significant *versus* control normal at P<0.05.
b: Significant *versus* glimipride-treated at P<0.05.

Table (2) shows adiponectin and dopamine in control patients and glimepride groups before and after supplementation with BC for 2 months. glimepride-treated patients showed significant increase (P<0.05) in serum adiponectin and dopamine levels compared to control patients. Administration of BC to glimepridegroup produced a significant increase in serum adiponectin level (P<0.05) compared to glimepride-treated patients (% increase 5.9%) but a more significant increase (P<0.05)in serum dopamine level compared toglimepride patients-before BC(% increase 35.9%).

Table 1: Adiponectin and dopamine levels of different studied participants at the end of the study

Parameter	Control group N=10	Glimipride-treated group N=15	GlimiprideBC group N=15	% increase
Adiponectin (µgm/mL)	7.1+1	41.3+6.9[a]	43.9+2.7[ab]	5.9
Dopamine	150+5.9 (nmol/24h)/10)	812.2+123.7[a] (nmol/L)	1267.8+602.3[ab] (nmol/L)	35.9

a: Significant *versus* control normal at P<0.05.
b: Significant *versus* glimipride-treated at P<0.05.

DISCUSSION

Metabolic characteristics of T2DM are indicated by distinct hyperglycemia, dyslipidemia, obesity and insulin resistance.Elevated insulin level could be a result of insulin resistance in peripheral tissues. Therefore, there is no specific cut-off value at which resistance begins and sensitivity ends. Consequently, insulin will be unable to act properly on resistant tissues and this resulted in poor glucose disposal and utilization.Compensatory hyperinsulinemia due to enhanced β-cell secretion was an obligate accompanying feature in insulin resistance(**Ebeid et al., 2012**). The main purpose of the antidiabetic therapies is to reduce and maintain glucose levels as close to normal and thereby prevent the development of complications. However, individual responses to these drugs can differ greatly, probably owing to the heterogeneous nature of the pathophysiology of T2DM (**Khalil et al., 2016**).

Glimepiride is a second-generation sulfonylurea acts directly by binding to the ATP-dependent potassium channels (K+ATP) on the β-cells. The closure of these channels by sulfonylureas results in depolarization of the β-cells and a successive calcium influx which leads to glucose-independent insulin release. Moreover, sulfonylureas are reported to inhibit glucagon secretion from pancreatic α-cells **(Bryan et al., 2005)**.We found that glimepiride had - to some extent glucose-lowering effect appeared in determined FBG levels. This finding was in accord with results from previous studies compared sitagliptinwith the sulfonylureaglibenclamide in T2DM patients. Glucose-utilizing effect of glimepiride contributed to lower HOMA-insulin resistance value in diabetic patients **(Saad et al., 2015)**.

Previous researches also represented that although initially effectivesulfonyl ureas in controlling hyperglycemia, theywere associated with poor durability, hypoglycemia and weight gain, possibly due to increased loss of β-cell function.Thisβ-cell function was found to be inversely proportional to failure rates using the homeostasis model assessment (HOMA-IR) **(DeFronzo, 2009; Ridderstrale et al., 2013)**. In our study, the insulin resistance index indicated that all diabetic subjects started the study with significantly higher HOMA-IR values compared to the control group. Otherwise, at the end of the treatment, all the treated patients showed a significant decline in the insulin resistance index by percentage of 70%in glimepride BC group compared to the patients treated with glimepride alone (56%).

Based on the support reported about the molecular and functional role of prolactin on human islets and islets insulin secretion **(Freemark, 2006; Yamamoto et al., 2008),** we recorded that, bromocriptine (BC) with glimepride at the administered doses resulted in lowering of glucose levels and improvement of the glycemic control in diabetic patients. Our results harmonized with recent results which demonstrate that pharmacological modulation of dopaminergic transmission by dopamine receptor D2   agonist BC tended to ameliorate insulin resistance in diet induced obesity animals **(Leminiet al., 2015)**. These data ensure that BC-mediated neurotransmission is involved in the control of glucose and insulin metabolism.

Our results alsoexhibited the main features of diabetic dyslipidemia in glimepride treated group compared to control healthy subjects: a high serum triglyceride, increased LDL-cholesterol, and low HDL cholesterollevels. The results of our study coincided with **Mooradianet al., 2009** who reviewedthat the increased flux of non-esterified fatty acids (NEFA)from adipocytes to liver directly affects insulin signaling, diminishes glucose uptake in muscle, exaggerates triglyceride synthesis, induces gluconeogenesis in the liver, and contributes to β-cell failure. Therefore, reducing serum NEFA concentration suppresses the driving force in insulin resistance and T2DM.

Co-administration of BC with glimepride exhibited a hypolipidmic action manifested by a significant decrease in T-C, LDL-C and TGs. Our findings were supported by other earlier studies; **Garber and colleagues (2013)** have reported that BC effect on CVD risk factors including lipid profile is more favorable in T2DM with history of CVD disease risk factors. Also, conducted two studies regarding the safety and efficacy of bromocriptin on major adverse cardiovascular events among patients with T2DM were taken place **(Gaziano *et al*., 2010;Gaziano *et al*., 2012)**.

A therapeutic strategy to modulate oxidative stress in diabetes is to exploit the pleiotropic properties of drugs directed primarily at other targets and thus acting as indirect antioxidants **(Tabatabaei-Malazy et al., 2015)**. Bromocriptine could be considered as one of these antioxidant drugs byprotecting LDL-C from peroxidation, a mechanism that could account for its cardioprotective action**(Karel et al., 2011)**.

Adipose tissue functions as an endocrine organ by secreting adipokines as well as storing triglycerides. It has been proven that adiponectin is under expressed in patients with T2DMwhich associated with obesity-related T2DM **(Henry et al., 2013)**. It is important to remember that sulfonyl ureas used to treat T2DM may induce weight gain, while BCis reported to be weight neutral.Our study indicated that glimepride treatment in uncontrolled T2DM significantly increaseadiponectine level when compared to control normal group. Previousresearches indicated that other antidiabetic drugs, but not glimepiride, at the administered doses significantly increased serum adiponectin level compared to the untreated diabetic controls**(Bray & Ryan, 2012)**.. The enhancement of endothelial function, reduction of cardiovascular risk associated with T2DM proved with antidiabetic drugs that increase adiponectin levels rather than glimepiride encourage us to try bromocriptin as adiponectin enhancer duringglimepride treatment.

In our study, it has been documented an improvement in weight after BC treatment. This could be explained when we know that prolactin is known to decrease adiponectin in both in vivo and in vitro which results in decrease in insulin sensitivity**(Pala et al., 2016)**. In addition.there was also nearly6 times rise in adiponectin levels in patients treated with bromocriptin compared to normal control subjects, indicating that this dopamine agonist may offer promise in the treatment of diabetes.

It is plausible that the presence in beta cells of the enzymes responsible for the synthesis, metabolization and storage of dopamine (TH, DOPA, MAO and VMAT-2) has been reported, it can be accepted that dopamine could be produced from beta cells and it would exert an auto-paracrine regulation of insulin secretion in these cells **(Brunetti &Kalabalik, 2012; Garcia et al., 2015)**. However, it has been speculated that

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the inhibition of glucose-stimulated insulin secretion induced by bromocriptinemay occur through alpha2-adrenergic receptors. Additionally, dopamine also act directly on dopamine receptors because the expression of D2, D3 and D4 dopaminergic receptors has been described in pancreatic islet cells**(Barrado et al., 2015)**.

Further using the theory that seasonal changes promote circadian neuroendocrine rhythms that play a role in insulin sensitivity and changes in body fat stores for preparation of hibernation or winter, dopaminergic and serotonergic activity is thought to contribute to this cycle and accompany insulin resistance. By delivering exogenous bromocriptine in the morning, a circadian resetting is thought to occur within the dopamine signals and produce a neurochemistry similar to a nondiabetic state **(Brian et al., 2014; Ralph et al., 2014)**

IV. Conclusion
In conclusion, bromocriptine concomitantly given with glimepride the second generation sulfonyl ureainvestigated to improve glycemic control in T2DM. These treatments remarkably ameliorated insulin resistance, suggested by a significant reduction inHOMA-IRvalue anda correction in lipidprofile. Furthermore, modulation of plasma adiponectin and dopamine may also underlie the improvement of insulin resistance and indicating that this dopamine agonist may offer promise in the treatment of diabetes.

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