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ORIGINAL ARTICLE

Targeting inflammation using celecoxib with glimepiride in the treatment of obese type 2 diabetic Egyptian patients

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Abstract Obesity, insulin resistance (IR), inflammation, and progressive decline in pancreatic β cell function are major features of type 2 diabetes mellitus (T2DM). We aimed to investigate the effect of co-administration of celecoxib (CE) with glimepiride (GL) in the treatment of obese T2DM patients. Body Mass Index (BMI), serum glucose, C-peptide, lipid profile, adiponectin, tumor necrosis factor-α (TNF-α), visfatin, and leptin levels were determined in 40 obese T2DM patients before and after treatment with GL alone or in combination with a selective cyclooxygenase-2 (COX-2) inhibitor CE for 3 months. Homeostasis model assessment of insulin resistance (HOMA2-IR) and atherogenic index (AI) was calculated. Increased levels of serum glucose, C-peptide, total cholesterol (TCH), low-density lipoprotein (LDL-C), triglycerides (TGs), visfatin, TNF-α, leptin, AI, and HOMA2-IR shown in obese diabetic patients were significantly decreased after co-treatment with GL plus CE compared to patients who received GL alone. On the other hand, adiponectin levels showed a significant increase after treatment. The obtained results demonstrate that targeting inflammation using celecoxib with glimepiride improves insulin resistance, glycemia, and inflammatory process in obese type 2 diabetics.

Keywords Visfatin · Celecoxib · Adiponectin · Leptin · T2DM · Obesity

Abbreviations

BG	Blood glucose
COPD	Chronic obstructive pulmonary disease
COX-2	Cyclooxygenase-2
CVD	Cardiovascular disease
ELISA	Enzyme-linked immunosorbent assay
GIT	Gastrointestinal tract
HDL-C	High-density lipoprotein
HOMA2-IR	Homeostasis model assessment of insulin resistance
HOMA2-β	Homeostasis model assessment of beta cell functionality
LDL-C	Low-density lipoprotein
T2DM	Type 2 diabetes mellitus
TCH	Total cholesterol
TGs	Triglycerides
TNF-α	Tumor necrosis factor alpha

Introduction

Diabetes is a complex, heterogeneous condition associated with beta cell dysfunction which is caused by many factors (e.g., hyperglycemia/glucotoxicity, lipotoxicity, autoimmunity, inflammation, adipocytokines, islet amyloid, incretins, and insulin resistance) [1]. Obesity is a chronic and low-grade inflammation that is involved in the pathogenesis of several chronic diseases, such as type 2 diabetes [2]. Inflammation is a physiological process characterized by elevated number of white

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blood cells and increased levels of pro-inflammatory cytokines in the circulation or tissue [3]. These proinflammatory cytokines, produced by adipose tissue, known as "adipocytokines" or "adipokines," include tumor necrosis factor-α (TNF-α), adiponectin, leptin, resistin, and visfatin [4]. Adipocytokines have been implicated as active participants in the development of insulin resistance and the increased risk of cardiovascular disease associated with obesity [5]. Chronic cyclooxygenase-2 (COX-2)-mediated inflammation seems to be involved in the development of insulin resistance in type 2 diabetes mellitus [6]. A previous study has demonstrated that COX-mediated inflammation and oxidative stress has been related to type 2 diabetes mellitus (T2DM) in elderly men [7]. Moreover, another report suggests that chronic COX-2-mediated inflammation in fat is crucial for obesity-linked insulin resistance [8, 9]. Alpert et al. has shown that the selective inhibitors of COX-2 augment the rate of glucose uptake to the plasma membrane in an insulin-independent manner [10]. The beneficial effect of COX-2 inhibitor on insulin resistance is partial via indirectly attenuating COX-2-mediated inflammation in adipose tissue [9]. On the other hand, Coll et al. demonstrated that COX-2 inhibition could enhance palmitate-induced inflammation in mouse [11]. However, the involvement of COX-2-mediated low-grade inflammation in the development of insulin resistance in obesity and T2DM remains controversial. Therefore, the present study was undertaken to examine the effect of co-administration of selective COX-2 inhibitors (celecoxib, CE) with oral hypoglycemic agent (glimepiride, GL) on the development of insulin resistance and increased inflammatory adipocytokines in obese diabetic Egyptian patients.

Subjects and methods

Patients

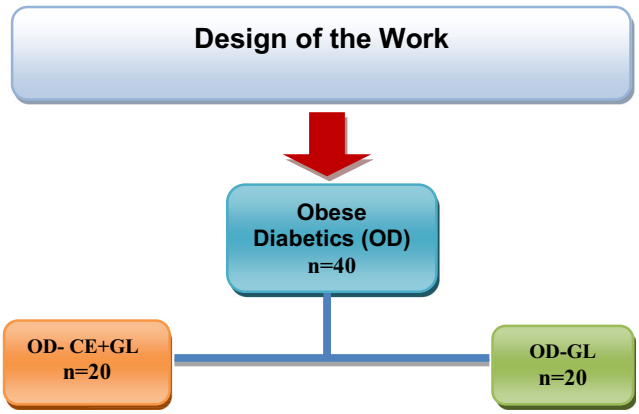
Forty obese diabetic patients were enrolled in a controlled random study from the Outpatient Clinics of Internal Medicine Department, Tanta University Hospitals, Tanta, Egypt. The protocol and potential risks and benefits of the study were fully explained to each subject before he or she provided their written informed consent. All experimental procedures followed the ethical standards and were approved by the Human Ethics Committees of Tanta University. Subjects were asked to maintain their usual dietary and physical activity habits throughout the study. All subjects' health status was evaluated by a complete medical examination. Obese diabetic patients (OD) (body mass index (BMI)≥30 kg/m^2) were randomly assigned into two groups (n=20 each) using a computer-generated random number: One group (OD-GL) received glimepiride (2 mg/day). The second group (OD-GL+CE) received glimepiride 2 mg/day plus celecoxib (100 mg twice daily) for 3 months.

Study design

Patients accepted in the present study were fulfilling the following criteria:

Inclusion criteria:

The selected diabetic patients control the serum glucose level with glimepiride for at least 3 months before the study. Age of the enrolled subjects, range from 30 to 65 years old. Glycated hemoglobin A1c % (HbA1c %) level ≤10 and fasting serum glucose levels between 130 and 300 mg/dl.



Exclusion criteria:

Subjects that were excluded from the study are those with type 1 diabetes mellitus, pregnant women, unstable cardiovascular disease (CVD), chronic obstructive pulmonary disease (COPD), impaired liver failure, impaired renal failure, smokers, findings of infection or autoimmune diseases or other chronic disease as determined by history, physical examination, and screening tests. Diabetic patients with diabetic complication were also excluded.

Assessment

Subjects' height and weight were recorded and BMI was calculated using the equation (BMI = weight (kg)/height (m)^2) [12]. Serum samples were obtained by centrifugation (10 min at 3500 r.p.m.) and immediately frozen at −20 °C until analysis. Fasting glucose determined using glucose oxidase method [13]. HbA1c % was determined by ion exchange method [14]. Serum visfatin [15], TNF-α [16], and adiponectin levels [17] were determined using enzyme-linked immunosorbent assay (ELISA) kits. Serum C-peptide quantified using immunoenzymometric assay kit [18]. Leptin serum level was determined using ELISA Kit [19]. Homeostasis model assessment (HOMA) was calculated using HOMA Calculator

version 2.2, where, C-peptide values are used [20]. Triglycerides (TGs) [21], total cholesterol (TCH), and high-density lipoprotein (HDL-C) [22] were measured calorimetrically. Low-density lipoprotein (LDL-C) was calculated [23]. Atherogenic index (AI) was calculated using the following ratio AI = log (TG)/HDL-C [24].

Statistical analysis

Data were analyzed using SPSS statistic 17.0 or Microsoft Excel 2010. Values are expressed as means±SD. Statistical analysis was performed according to the repeated measurements of one-way analysis of variance (ANOVA) followed by LSD as post hoc test. A probability $P<0.05$ was considered statistically significant.

Results

All the patients completed the study with no major side effects. The only minor side effects were tinnitus and mild gastrointestinal tract (GIT) disturbances, which tended to be decreased with continuation of the treatment and disappeared completely after stopping of celecoxib. In the present study, BMI was not changed after 3 months of treatment in both groups. Our results showed that fasting serum blood glucose (BG), C-peptide, and HbA1c % significantly decreased after treatment with GL+CE compared to treatment with GL alone. HOMA2-IR significantly reduced after treatment with GL+CE compared to treatment with GL alone. On the other hand, HOMA2-β % values significantly increased after GL+CE treatment compared to GL treatment (Table 1).

Serum adiponectin level increased significantly after 3 months of treatment with a significant difference when comparing group the GL group with the GL+CE group. On the other hand, serum visfatin and leptin concentrations significantly decreased after 3 months of treatment in both groups, with no difference when comparing the GL group to the GL+CE group. Serum TNF-α level decreased significantly after treatment in both groups with a significant difference comparing group received GL alone to group received GL+CE. TCH and LDL-C levels were not changed in patients who received GL, whereas significantly decreased in patients who received GL+CE treatment, with a significant difference when comparing OD-GL and OD-GL+CE groups. Serum HDL level exhibited no change in both groups. Triglyceride TGs level showed significant decrease upon treatment and when comparing the OD-GL+CE and OD-GL groups, it was found a significant difference. On the other hand, AI decreased significantly in OD-GL+CE group compared to before treatment with a significant difference compared to OD-GL group (Table 2).

Discussion

The present study evaluate whether the co-administration of anti-inflammatory agent celecoxib with glimepiride could have an impact on glycemia, insulin resistance, and adipocytokines levels in obese diabetic subjects. The hallmark of obesity and type 2 diabetes is insulin resistance, a result of decreased insulin metabolic signaling due to enhanced serine phosphorylation and/or degradation of the insulin receptor substrate [25]. The major factors for progressive loss of beta cell function and mass are glucotoxicity, lipotoxicity, proinflammatory cytokines, leptin, and islet cell amyloid. Impaired beta cell function possibly appears to be reversible, particularly at early stages of the disease [26]. Our results implicate that COX-2 inhibition could be a therapeutic drug for treatment of obese subjects with T2DM and may prevent associated complications by suppressing the increased level of adipocytokines and insulin resistance in OD patients. The obtained results showed that fasting serum BG, C-peptide, HbA1c %, and HOMA of insulin resistance (HOMA2-IR) significantly decreased after treatment with GL+CE compared to treatment with GL alone. HOMA of beta cell functionality (HOMA2-β %) values significantly increased after GL+CE treatment compared to GL treatment. In consistence with a recent study that examined the effect of COX-2 inhibition on the development of muscular insulin resistance in high-fat-induced obese rats, emphasized that chronic COX-2 inhibition could significantly suppress insulin resistance via other mechanism than its direct action [27]. Previous study demonstrated that COX-2 inhibition significantly suppressed the whole body and muscular insulin resistance, implying the importance of COX-2-mediated low-grade inflammation in the pathogenesis of insulin resistance in metabolic syndrome and T2DM [28]. These observations indicate that COX-2-mediated inflammatory response might be an important cause of the development of insulin resistance in T2DM and obesity. Nevertheless, some case reports showed that COX-2 inhibitors could induce hypoglycemic episode when over consumed or taken in combination with oral hypoglycemic drugs [29, 30]. Recent study suggested that the changes in COX activity are involved in the regulation of glucose homeostasis under the states of normal and insulin resistance [31]. The present study showed that serum adiponectin was significantly increased whereas, serum visfatin, leptin, and TNF-α levels were significantly decreased after co-administration of CE with GL. Adiponectin and TNF-α are important inflammatory products with potential involvement in the pathogenesis of tissue insulin resistance [32]. Consistent with the present finding, Hsieh et al. have shown that the COX-2 inhibitors could significantly reverse the enhanced TNF-α and the decreased adiponectin as well as in obese rats [9]. Furthermore, the present results showed that the elevated serum values of TC, LDL-C, TGs, and AI in obese diabetics were corrected with

Table 1 Effect of treatment on BMI, blood glucose, glycated hemoglobin, C-peptide, and HOMA levels

Group	OD-GL		OD-GL+CE	
Parameter	B	A	B	A
Age	48±5.52	–	51.3±6.28	–
Gender (F/M)	11/9	–	10/10	–
BMI	32.93±1.97	32.82±1.96	33.12±1.61	32.90±1.56
FBG mg/dl	186.25^{b}±13.80	177.05^{ab}±16.05	181.95^{b}±16.97	136.8^{a}±19.83
HbA1c %	8.11^{b}±0.45	7.71^{ab}±0.76	8.01^{b}±0.52	7.05±0.44
C-peptide ng/ml	1.91^{b}±0.30	1.80^{ab}±0.25	1.96^{b}±0.33	1.58^{a}±0.31
HOMA2-IR	1.77^{b}±0.28	1.64^{ab}±0.23	1.81^{b}±0.31	1.33^{a}±0.28
HOMA2-β %	32.30^{b}±5.78	33.84^{b}±6.89	34.4^{b}±7.32	50.75^{a}±18.13

Data are presented as mean±SD; $P<0.05$

A after treatment, *B* before treatment, *SD* standard deviation, *BMI* body mass index, *FBG* fasting blood glucose, *HbA1c* % glycated hemoglobin percent, *HOMA2-IR* homeostasis model assessment of insulin resistance, *HOMA2*-β homeostasis model assessment of beta cell functionality, *OD-GL* obese diabetic group treated with glimepiride drug, *OD-GL+CE* obese diabetic group treated with glimepiride plus celecoxib drugs, *F* female, *M* male

[a] Significant before versus after treatment

[b] Significant versus after treatment with GL+CE

combination treatment of celecoxib and glimepiride. Accumulation of muscle TGs has been proposed to inversely relate with the defective glucose uptake in insulin-resistant subjects [33]. Jeuestte et al.[34] proposed that insulin resistance in obese diabetic subjects may be responsible for enhanced overproduction of TGs and cholesterol-rich lipoprotein by liver. Pro-inflammatory molecules produced by adipose tissue have been implicated as active participants in the development of insulin resistance [5]. This is explained by the increased oxidative stress, which could originate from adipose tissue in obesity, possibly leads to impaired insulin production and insulin action [31]. Taken together, these results implicate that COX-2 mediated generation of oxidative stress might play an important role in the development of obesity and T2DM in humans. COX-2 inhibition could be a therapeutic drug target to treat obese subjects with T2DM and prevent complications.

Table 2 Effect of treatment on visfatin, adiponectin, leptin, TNF-α levels, and lipid profile

Group	OD-GL		OD-GL+CE	
Parameter	B	A	B	A
Adiponectin (pg/ml)	0.55^{b}±0.09	1.17^{ab}±0.38	0.57^{b}±0.11	1.80^{a}±0.51
Visfatin (ng/ml)	91.69^{b}±16.64	84.33^{a}±15.41	91.09^{b}±12.00	77.18^{a}±11.90
Leptin (ng/ml)	11.96^{b}±2.03	11.02^{a}±2.12	12.17^{b}±3.43	9.34^{a}±4.18
TNF-α (pg/ml)	1.91^{b}±0.43	1.70^{ab}±0.42	1.93^{b}±0.30	1.33^{a}±0.29
Total cholesterol mg\dl	196.31^{b}±19.38	191.66^{b}±19.24	194.18^{b}±14.66	177.75^{a}±12.82
LDL-C mg/dl	129.02^{b}+19.35	125.63^{b}+19.95	126.68^{b}+13.56	117.47^{a}+15.31
HDL-C mg/dl	32.82±1.25	32.98±1.23	32.75±1.25	33.16±1.37
Triglycerides mg/dl	172.36^{b}+14.13	165.25^{ab}±11.75	173.73^{b}+16.39	135.65^{a}±22.41
AI	0.72^{b}±0.04	0.70^{b}±0.04	0.72^{b}±0.05	0.61^{a}±0.07

Data are presented as mean±SD. $P<0.05$

A after treatment, *B* before treatment, *TNF-α* tumor necrosis alpha, *LDL-C* low-density lipoprotein cholesterol, *HDL-C* high-density lipoprotein cholesterol, *AI* atherogenic index, *OD-GL* obese diabetic group treated with glimepiride drug, *OD-GL+CE* obese diabetic group treated with glimepiride plus celecoxib drug

[a] Significant before versus after treatment

[b] Significant versus after treatment with GL+CE

The present study demonstrates that COX-2 inhibition could alleviate obesity-induced insulin resistance indirectly via suppressing adipocytokines released in obese diabetics.

Study limitations

Limitations of the study are the small number of participated patients and as with all NSAIDs, the potential GIT, CVD, and renal risks of CE must be weighed against the potential benefits in each individual.

Conclusions

Increased insulin resistance in obese diabetics due to increased inflammatory adipocytokines visfatin, leptin, and TNF-α and decreased adiponectin levels can be reversed by co-treatment with COX-2 inhibitor (celecoxib). So, COX-2 inhibition could be a therapeutic drug target for treatment of obese subjects with T2DM and prevent or delay complications. More extensive studies are needed to evaluate the use of anti-inflammatory strategies, especially COX-2 inhibitors, as preventative and therapeutic interventions in obesity and type 2 diabetes.

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Conflict of interest The authors declared that there are no conflicts of interest.

Author's contribution Hoda El-Bahrawy did the conception and design of the study and final approval of the version to be published. Sahar Hegazy followed up the patients and drafted the article or revised it critically for important intellectual content. Wael Farrag took the detailed history from patients, examined the patients clinically, and analyzed and interpreted the data. Rehab Werida followed up the patients and collected samples from them; performed anthropometric evaluations and biochemical assay for selected parameters; analyzed obtained data; and drafted the article or revised it critically for important intellectual content, and gave final approval of the version to be published.

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