Curcumin/Irbesartan Combination Improves Insulin Sensitivity and Ameliorates Diabetes-Induced Pro-Inflammatory Cytokines in Type-2 Diabetes Rat Model

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Abstract

Objectives: This study aimed to evaluate the impact of chronic administration of an angiotensin II receptor antagonist (Irbesartan) and/or curcumin on blood glucose level, insulin sensitivity, and proinflammatory cytokines in experimentally-induced type-2 diabetes in albino rats.

Material and Methods: The study comprised 50 male albino rats; 20 rats as control group and 30 rats were maintained on high-fat diet for 2-weeks and had induced non-insulin dependent diabetes mellitus (NIDDM) using intraperitoneal injection of a single dose of streptozotocin (STZ) in a dose of 50 mg/kg and 1-week later, rats were subdivided into three equal subgroups received oral irbesartan (2.5 mg/kg/day), oral curcumin (200 mg/kg) and both lines in that defined doses, respectively, for 6 weeks. Homeostasis Model Assessment of Insulin Resistance (HOMA-IR) was used for clinical assessment. Two fasting venous blood samples were obtained after induction of diabetes and prior to initiation of therapy and at 6-wks after treatment for estimation of fasting insulin (FI), fasting blood glucose (FBG), serum interleukin (IL)-1β and IL-6 and tumor necrosis factor-α (TNF-α).

Results: Both lines of treatment either alone or in combination induced significant reduction of FBG and FI levels compared to their pre-treatment levels and administration of curcumin either alone or in combination with irbesartan induced significant reduction of FBG compared to irbesartan, but combination therapy significantly lower FI levels compared to animals either irbesartan or curcumin alone. Post-treatment HOMA-IR indices were significantly improved in the studied subgroups compared to pre-treatment levels, with the effect was more significantly pronounced with the used combination of curcumin and irbesartan. Furthermore, post-treatment serum levels of studied cytokines were significantly lower compared to pre-treatment levels, irrespective of line of treatment applied and administration of curcumin, either alone or in combination with irbesartan significantly reduced serum levels of IL-6 and TNF-α compared to irbesartan alone.

Conclusion: Chronic administration of irbesartan/curdemin combination showed anti-diabetic effect manifested as decreased FBG and FI levels and ameliorated the increased serum levels of pro-inflammatory cytokines. The use of such combination could be recommended for clinical trials so as to document its use for control of type-2 diabetes.

Key Words: Diabetes type 2 albino rats – Insulin sensitivity – Ameliorates diabetes – Pro-inflammatory cytokines – Curcumin.

Introduction

INSULIN resistance is typically defined as decreased sensitivity or responsiveness to metabolic actions of insulin, such as insulin-mediated glucose disposal in skeletal muscle and adipose tissue and inhibition of hepatic glucose production [1]. Cross-talk between inflammatory signaling pathways and insulin signaling pathways causes both metabolic insulin resistance and endothelial dysfunction [2].

Insulin resistance plays a major pathophysiological role in type 2 diabetes and is tightly associated with major public health problems, including obesity, hypertension, coronary artery disease, dyslipidemias, and a cluster of metabolic and cardiovascular abnormalities that define the metabolic syndrome [3]. The metabolic syndrome is considered to be a proinflammatory state because it is associated with elevated levels of high-sensitivity C-reactive protein, IL-6, fibrinogen, and plasminogen activator inhibitor-1, all of which promote the development of atherosclerotic cardiovascular disease [4]. Therefore, improvement of insulin sensitivity is an important therapeutic goal.

Improvement of insulin sensitivity has been suggested in many reports to be feasible by certain herbs and drugs. For instance, it was reported that
Curcumin/Irbesartan Combination Improves Insulin Sensitivity

Curcumin improve blood glucose and insulin sensitivity in rat models of diabetes [5]. Curcumin, a polyphenolic compound, is the major yellow-colored pigment found in the spice, turmeric. It has been used in traditional Indian medicine for centuries, and has numerous pharmacological activities, including potent anti-inflammatory, antioxidant, chemopreventive and chemotherapeutic actions [6].

Angiotensin II (Ang II), the main effector peptide of the renin–angiotensin system (RAS), is implicated in the development of vascular, cardiac, and renal pathologies. Several lines of evidence suggest that Ang II impairs insulin sensitivity and provoke glucose intolerance [7,8]. Furthermore, angiotensin type-1 receptor (AT1R) blockers (ARBs) have recently been demonstrated to exert beneficial effects on glucose and lipid metabolism in adipocytes and adipose tissue [9]. The RAS by blockade of the AT 1 R substantially lowers the risk for type 2 diabetes [10]. Additionally, blockade of the AT 1 R has been shown to improve insulin sensitivity in animal models of insulin resistance [11]. However, the mechanisms underlying the insulin-sensitizing and antidiabetic effects of the ARBs have not been defined. Findings from in vitro and in vivo studies have revealed that two newer ARBs, telmisartan and irbesartan, have the potential to improve insulin sensitivity and beta-cell responsiveness [12].

The present study was designed to evaluate the impact of chronic administration of an angiotensin II receptor antagonist (Irbesartan) and/or curcumin on blood glucose level, insulin sensitivity, and proinflammatory cytokines in experimentally-induced type-2 diabetes in albino rats.

Material and Methods

Animals:

The present study comprised 50 male albino rats with weight range of 250-300 grams. Rats were grouped and kept in separate animal cages, under the prevailing atmospheric conditions and maintained on a balanced diet and fresh-water supply.

Induction of diabetes:

Type 2 diabetes mellitus (NIDDM group) was induced by feeding rats with high-fat diet (HFD) consisting of 22% fat, 48% carbohydrate and 20% protein. After two weeks, rats were injected intraperitoneally with a single dose of streptozotocin (STZ) (Sigma) in a dose of 50 mg/kg body weight dissolved in 0.2 ml of citrate buffer (pH 4.5) [13].

On the third day of injection, the animals were checked for the presence of glucose in the urine using enzymatic test strips as STZ induces diabetes within 3 days by destroying the beta cells [14]. Confirmation was done by measuring fasting blood glucose levels by taking a drop of blood from the rat-tail using a glucose-measuring device (Glucocheck). Rats had blood glucose levels of ≥ 200 mg/dl were considered diabetic [13].

Grouping & dosing:

Studied animals were divided into two main groups:

Group I (Control group): 20 animals were considered as a control group for estimated parameters and were divided into two subgroups:

Group I-A: Included 10 rats received no medications and kept under the same conditions as prior to start of the study.

Group I-B: Included 10 rats were injected intraperitoneally with one injection of citrate buffer and received 1ml/rat of 1% gum acacia orally for 6 weeks.

Group II (NIDDM group): 30 rats had induced non-insulin dependent diabetes mellitus (NIDDM). One week after induction of diabetes, rats were subdivided into three equal subgroups:

Group II-Irb: 10 rats were administered irbesartan in a dose of 2.5 mg/kg/day orally for a period of 6 weeks.

Group II-Cur: 10 rats were administered 200 mg/kg body weight of curcumin orally/day for a period of 6 weeks.

Group II-Irb/Cur: 10 rats were administered both irbesartan in a dose of 2.5 mg/ kg/day orally and curcumin in a dose of 200 mg/kg body weight orally/day for a period of 6 weeks.

Irbesartan (Sanofi-Aventis) and curcumin (Sigma chemicals) pure powder were suspended in 1% gum acacia so that 0.5 to 1 ml contained the desired dose. The therapeutic human dose of irbesartan was converted to rat dose according to Paget converting table [15] and about half of the therapeutic dose was used in this study.

Homeostasis Model Assessment of Insulin Resistance (HOMA-IR) [16]: This test was used for insulin sensitivity evaluation on the basis of fasting insulin and glucose levels and according to the formula HOMA-IR= I x G/22.5, where I is fasting...
insulin level (µIU/ml) and G is fasting blood glucose in mg/dl divided by [18,17].

Biochemical evaluation:

Two fasting venous blood samples, withdrawn from the tail vein, were obtained, the 1st after induction of diabetes and prior to initiation of therapy and the 2nd at the end of the 6-wks treatment period. Blood samples were divided into 2 parts:

The first was put in a tube containing sodium fluoride (2 mg sodium fluoride/ml blood) to prevent glycolysis. Plasma was separated by centrifugation and used for estimation of glucose by glucose oxidase method [18].

The second part was allowed to clot then serum was separated by centrifugation at 3000 rpm for 10 min. Serum was removed, divided into 2 parts: the first for RIA determination of serum level of insulin [19] and the second part was placed in pyrogen-free Eppendorf tubes and stored at –80 °C until ELISA assayed (within one month) for estimation of serum levels of IL-1β, [20], IL-6, [21] and TNF-α, [22] using Quantikine ELISA kits from R & D Systems, Inc., (Minneapolis, MN).

Statistical analysis:

Obtained data were presented as mean ± SD and ranges, were analyzed using paired t-test. Statistical analysis was conducted using the SPSS (Version 10, 2002) for Windows statistical package. p value <0.05 was considered statistically significant.

Results

Estimated variables showed a non-significant (p>0.05) difference between both subgroups of control rats, (Table 1), so all statistical analysis of study groups were conducted versus control group I-A that arbitrary named control group.

Fasting blood glucose levels estimated either prior to or at end of therapy, were significantly higher in all studied animals compared to control levels. Both lines of treatment either alone or in combination induced significant reduction of FBG levels compared to pre-treatment levels. However, administration of curcumin in combination with irbesartan induced significant reduction of FBG compared to either drug alone (Table 2, Fig. 1).

Fasting insulin (FI) levels estimated either prior to or at end of therapy, were significantly higher in group II animals compared to control group. Post-treatment FI levels were significantly lower compared to pre-treatment levels in all studied animals, but animals administered combination of irbesartan and curcumin showed significantly lower FI levels compared to animals received either irbesartan or curcumin alone with a significantly lower FI levels in animals received irbesartan compared to those received curcumin alone (Table 2, Fig. 2).

HOMA-IR index calculated prior to initiation of therapy was significantly higher in studied subgroups compared to control index with non-significant difference among these subgroups. Post-treatment HOMA-IR index was significantly decreased in the three subgroups compared to pre-treatment levels, despite still being significantly higher compared to control group. Combination of irbesartan and curcumin significantly reduced HOMA-IR index compared to either irbesartan or curcumin alone with a non-significant difference in favor of irbesartan (Table 2, Fig. 3).

Pre-treatment levels of studied pro-inflammatory cytokines were significantly higher compared to control level. Post-treatment serum levels of IL-1β were significantly higher in animals administered irbesartan alone or curcumin alone, but was non-significantly higher in animals administered irbesartan/curcumin combination compared to control level, despite being significantly lower compared to pre-treatment levels, irrespective of line of treatment. Moreover, serum IL-1β showed a non-significant difference between treated animals, despite being lower in combination group. Moreover, administration of curcumin, either alone or in combination with irbesartan significantly reduced serum IL-6 in comparison to irbesartan alone with non-significantly lower levels in combination group compared to curcumin group, (Fig. 4). As regards post-treatment serum TNF-α, combination groups showed significantly lower levels compared to irbesartan alone and non-significantly lower levels compared to curcumin (Table 3, Fig. 5).

Table (1): Mean values estimated in both control subgroups.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Group I-A</th>
<th>Group I-B</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fasting blood glucose (mg/dl)</td>
<td>77.4±9.1</td>
<td>81±9.3</td>
</tr>
<tr>
<td>Fasting insulin (µIU/ml)</td>
<td>0.9±0.2</td>
<td>0.82±0.21</td>
</tr>
<tr>
<td>HOMA-IR index</td>
<td>0.17±0.03</td>
<td>0.16±0.05</td>
</tr>
<tr>
<td>IL-1β (pg/ml)</td>
<td>1.28±0.23</td>
<td>1.19±0.31</td>
</tr>
<tr>
<td>IL-6 (pg/ml)</td>
<td>12.2±3.3</td>
<td>11.9±4.2</td>
</tr>
<tr>
<td>TNF-α (pg/ml)</td>
<td>1.82±0.6</td>
<td>1.86±0.52</td>
</tr>
</tbody>
</table>
Table (2): Mean (±SD) of FBG and FI levels and HOMA-IR index estimated in NIDDM animals pre- and post-treatment compared to control levels.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Group</th>
<th>Control</th>
<th>NIDDM Irb</th>
<th>NIDDM Cur</th>
<th>NIDDM Irb/Cur</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Irb</td>
<td>Cur</td>
<td>Irb/Cur</td>
</tr>
<tr>
<td>Fasting blood glucose (mg/dl)</td>
<td>Pre-ttt</td>
<td>77.4±9.1</td>
<td>251.7±32.4*</td>
<td>259.9±35.7*</td>
<td>273.3±38.6*</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>172.3±11.2**↑</td>
<td>160.7±18.1**↑</td>
<td>155.7±19.4**↑††</td>
</tr>
<tr>
<td></td>
<td>Post-ttt</td>
<td></td>
<td>251.7±32.4*</td>
<td>259.9±35.7*</td>
<td>273.3±38.6*</td>
</tr>
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<td></td>
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<td></td>
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<td>160.7±18.1**↑</td>
<td>155.7±19.4**↑††</td>
</tr>
<tr>
<td>Fasting insulin (IU/ml)</td>
<td>Pre-ttt</td>
<td>0.9±0.21</td>
<td>4.9±1*</td>
<td>5.1±1.3*</td>
<td>5.28±1.2*</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>3.14±0.41*↑†</td>
<td>3.61±0.35*↑</td>
<td>2.55±0.54*↑††</td>
</tr>
<tr>
<td></td>
<td>Post-ttt</td>
<td></td>
<td>0.9±0.21</td>
<td>4.9±1*</td>
<td>5.1±1.3*</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>3.14±0.41*↑†</td>
<td>3.61±0.35*↑</td>
<td>2.55±0.54*↑††</td>
</tr>
<tr>
<td>HOMA-IR index</td>
<td>Pre-ttt</td>
<td>0.17±0.03</td>
<td>3.07±0.84*</td>
<td>3.28±0.93*</td>
<td>3.54±0.84*</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>1.33±0.18*↑</td>
<td>1.43±0.2*↑</td>
<td>0.99±0.27*↑††</td>
</tr>
<tr>
<td></td>
<td>Post-ttt</td>
<td></td>
<td>0.17±0.03</td>
<td>3.07±0.84*</td>
<td>3.28±0.93*</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>1.33±0.18*↑</td>
<td>1.43±0.2*↑</td>
<td>0.99±0.27*↑††</td>
</tr>
</tbody>
</table>


Table (3): Mean (±SD) of serum levels of IL-1β, IL-6 and TNF-α estimated in studied animals pre- and post-treatment compared to control levels.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Group</th>
<th>Control</th>
<th>NIDDM Irb</th>
<th>NIDDM Cur</th>
<th>NIDDM Irb/Cur</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Irb</td>
<td>Cur</td>
<td>Irb/Cur</td>
</tr>
<tr>
<td>IL-1β (pg/ml)</td>
<td>Pre-ttt</td>
<td>1.28±0.23</td>
<td>2.29±0.3*</td>
<td>2.14±0.51*</td>
<td>2.22±0.46*</td>
</tr>
<tr>
<td></td>
<td>Post-ttt</td>
<td></td>
<td>1.2±0.41*↑</td>
<td>1.58±0.4*↑</td>
<td>1.4±0.32↑</td>
</tr>
<tr>
<td>IL-6 (pg/ml)</td>
<td>Pre-ttt</td>
<td>12.2±3.3</td>
<td>40.7±14.1*</td>
<td>43.9±11.6*</td>
<td>43.2±9.3*</td>
</tr>
<tr>
<td></td>
<td>Post-ttt</td>
<td></td>
<td>28.5±1.9*↑</td>
<td>24±2.3*↑↑</td>
<td>21.9±2.3*↑↑</td>
</tr>
<tr>
<td>TNF-α (pg/ml)</td>
<td>Pre-ttt</td>
<td>1.82±0.6</td>
<td>6.86±1.8*</td>
<td>6.4±1.9*</td>
<td>6.7±2*</td>
</tr>
<tr>
<td></td>
<td>Post-ttt</td>
<td></td>
<td>3.82±0.7*↑</td>
<td>3.6±0.8*↑</td>
<td>3.4±0.6*↑↑</td>
</tr>
</tbody>
</table>


Fig. (1): Mean pre and post-treatment FBG levels in NIDDM animals.

Fig. (2): Mean pre- and post-treatment FI (IU/ml) estimated in NIDDM groups.
Discussion

Type-2 diabetes is a kind of heterogeneous disease with complicated pathogenesis which is related to genetic susceptibility and life style, especially the dietetic style. The establishment of its experimental model will be helpful in understanding its pathogenesis and the development of new treatments [23].

The present study was designed to evaluate the impact of chronic administration of an angiotensin II receptor antagonist (Irbesartan) and/or curcumin on insulin sensitivity in experimentally-induced NIDDM animal models. Both lines of treatment either alone or in combination induced significant reduction of FBG and FI levels compared to their pre-treatment levels and administration of curcumin either alone or in combination with irbesartan induced significant reduction of FBG compared to irbesartan, but combination therapy significantly lower FI levels compared to animals received either irbesartan or curcumin alone.

These findings spot line on the fact that both of curcumin and irbesartan induced lowering of FBG by a different mode of action towards one target, i.e. lowering FBG and both could act synergistically and that the effect of the studied drugs was conducted through increasing the sensitivity of insulin receptor to the available secreted amount of insulin and consequently increased glucose metabolism with lowering FBG without any impact on insulin secretion.

These findings coincided with and supported that previously reported by Pari & Murugan [24] who investigated the effect of tetrahydrocurcumin (THC), one of the active metabolites in curcumin, on the key hepatic metabolic enzymes involved in carbohydrate metabolism in STZ-induced diabetic rats and found that in untreated diabetic control rats, the activities of the gluconeogenic enzymes were significantly increased, whereas hexokinase and glucose-6-phosphate dehydrogenase activity and glycogen levels were significantly decreased, while both THC and curcumin were able to restore the altered enzyme activities to near normal levels and normalize blood glucose in diabetic rats. Also, Murugan & Pari [25] investigated the effect of THC on lipid profile and lipid peroxidation in type-2 diabetic rats and reported a significant reduction in blood glucose, which proved its antidiabetic effect and caused a significant reduction in lipid peroxidation and lipids in serum and tissues, suggesting its role in protection against lipid peroxidation and its antihyperlipidemic effect.
Thereafter, Murugan & Pari [26] and Suryanarayana et al. [27] examined the effect of THC and curcumin on erythrocyte membrane bound enzymes and antioxidants activity in type-2 diabetic model and reported that administration of THC and curcumin induced increased levels erythrocyte antioxidants and the activities of membrane bound enzymes and concluded that these biochemical observations indicate that the THC and curcumin possess a significant beneficial effect on erythrocyte membrane bound enzymes and antioxidants defense in addition to its antidiabetic effect.

Niu et al. [28] investigated the role of angiotensin-converting enzyme 2 (ACE2) in regulating glucose homeostasis and found that ACE2 was positively expressed in the pancreas and male ACE2 knockout mice displayed a selective decrease in first-phase insulin secretion in response to glucose and a progressive impairment of glucose tolerance and concluded that ACE2 might play an important role in glucose homeostasis as well as type 2 diabetes.

In support of the reported data, post-treatment HOMA-IR indices were significantly improved in the studied subgroups compared to pre-treatment levels, with the effect was more significantly pronounced with the used combination of curcumin and irbesartan. Such clinical implication of the obtained results goes in hand with various clinical studies; Huang et al. [29] suggested that a local pancreatic renin-angiotensin system and pravastatin, captopril and irbesartan treatment may be selectively controlling pancreatic islet blood flow, augmenting insulin secretion and thereby improving glucose tolerance and concluded that the antidiabetic actions of renin-angiotensin system inhibitors might occur, in part, through the beneficial direct islet effects. Cetinkalp et al. [30] found the short-term treatment of irbesartan is effective to decrease microalbuminuria in normotensive type-2 diabetes patients independent of its antihypertensive effect and such decrease was associated with significantly decreased fasting and non-fasting blood glucose, and glycosylated haemoglobin (HbA1c) compared to pre-treatment values. These beneficial effects could be attributed to the previously reported that chronic administration of ACE inhibitors to obese rats results in a significant diminution of the elevated level of fasting plasma free fatty acids and this decrease in free fatty acids may be mechanistically linked to the improvement in insulin action on skeletal-muscle glucose transport (GLUT4) [31, 32].

Furthermore, post-treatment serum levels of studied cytokines were significantly lower compared to pre-treatment levels, irrespective of line of treatment applied and administration of curcumin, either alone or in combination with irbesartan significantly reduced serum levels of IL-6 and TNF-a compared to irbesartan alone. Such ameliorative effect of curcumin and irbesartan administered separately or in combination on pro-inflammatory cytokines could be a possible mechanism for the reported effects on insulin sensitivity that proved to be improved irrespective of the drug used.

These data go in hand with Ceriello et al. [33] who reported an independent and cumulative effect of postprandial hypertriglyceridemia and hyperglycemia on endothelial function and inflammation, suggesting oxidative stress as a common mediator of such an effect and short-term treatment with irbesartan may counterbalance this phenomenon. Also, Persson et al. [34] evaluated the impact of irbesartan treatment on biomarkers of low-grade inflammation in patients with type 2 diabetes and microalbuminuria and found irbesartan treatment yielded significant changes in CRP with a 5.4% decrease per year versus a 10% increase per year in the placebo group. IL-6 showed a 1.8% increase per year compared with placebo's 6.5% increase per year and changes in IL-6 were associated with changes in albumin excretion and concluded that irbesartan reduces low-grade inflammation in this high-risk population. Vieitez et al. [35] found systemic and local administration of irbesartan lowers glomerular expression of growth factors and TNF-α and concluded that part of the effect of lowering the expression of these growth factors and cytokines is due to a direct blockade of glomerular renin-angiotensin system.

It could be concluded that chronic administration of irbesartan/curcumin combination showed anti-diabetic effect manifested as decreased FBG and FI levels and ameliorated the increased serum levels of pro-inflammatory cytokines. The use of such combination could be recommended for clinical trials so as to document its use for control of type-2 diabetes.

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