Effects of rosiglitazone and aspirin on experimental model of induced type 2 diabetes in rats: focus on insulin resistance and inflammatory markers

Amany A. Abdin⁎, Amal A. Baalashb, Hala E. Hamooda

⁎Department of Pharmacology, Tanta Faculty of Medicine, Tanta, Egypt
bDepartment of Medical Biochemistry, Tanta Faculty of Medicine, Tanta, Egypt

Received 31 January 2008; accepted 21 January 2009

Abstract

Both insulin resistance and decreased insulin secretion are major features of the pathophysiology of type 2 diabetes. Inflammatory pathways are found to be critical in mechanisms underlying insulin resistance, which is a major determinant of increased risk of cardiovascular complications in type 2 diabetes, and so, it is a potential therapeutic target. Thiazolidinediones (e.g., rosiglitazone) act primarily as insulin sensitizers and were discovered to have anti-inflammatory effects leading to reevaluation of their potential use in treatment of diabetes. Acetyl salicylic acid (aspirin), which is currently recommended for cardiovascular disease (CVD) or even CVD risk factors, is shown to ameliorate diabetic process. This work aimed to study correlation between homeostasis model assessment estimate of insulin resistance (HOMA-IR) with serum levels of inflammatory markers tumor necrosis factor α (TNF-α), interleukin 6 (IL-6), C-reactive protein (CRP), and free fatty acids (FFAs) in experimental model of induced type 2 diabetes in rats, with evaluation of effects of rosiglitazone and aspirin (low or high dose), alone or in combination. There is significant elevation of insulin resistance and serum levels of fasting glucose, insulin, TNF-α, IL-6, CRP, and FFAs in the diabetic group when compared to the normal group, with positive significant correlation between levels of each of TNF-α, IL-6, CRP, and FFAs with insulin resistance (HOMA-IR). Administration of rosiglitazone, low-dose aspirin, or high-dose aspirin to diabetic rats caused nonsignificant lowering in insulin level with significant reduction of levels of other parameters when compared to the diabetic group. Also, there is no significant difference in the measured parameters between diabetic rats administered a combination of rosiglitazone with high-dose aspirin and those administered a combination of rosiglitazone with low-dose aspirin. It was concluded that aspirin and rosiglitazone offer unique approaches for treatment of type 2 diabetes due to their insulin-sensitizing and anti-inflammatory properties, and their combination was found to provide augmented beneficial effects. Also, in view of the potential dose-dependent adverse effects of aspirin, with no achievement of further benefit by high dose in this study, it is strongly recommended to use low-dose aspirin as a safe and effective medication for diabetes.

© 2010 Elsevier Inc. All rights reserved.

Keywords: Type 2 diabetes; Rosiglitazone; Aspirin; Insulin resistance; Inflammatory markers

1. Introduction

Both insulin resistance and decreased insulin secretion are major features of the pathophysiology of type 2 diabetes (non-insulin-dependent diabetes mellitus) (Evans, Goldfine, Maddux, & Grodsky, 2003).

Insulin resistance is defined as a decreased response of the peripheral tissues to insulin action (Xu et al., 2003), and it most often precedes the onset of hyperglycemia and predicts development of type 2 diabetes (Saad et al., 1989; Yki-Jarvinen, 1994).

Several studies left little doubt that inflammatory pathways are critical in the mechanisms underlying insulin resistance and type 2 diabetes, resulting in deteriorated metabolic homeostasis in general and glucose metabolism in particular (Wellen & Hotamisligil, 2003); where even minimal disturbances in glucose tolerance are associated with a chronic, generalized inflammatory reaction that links components of the metabolic syndrome and contributes to

⁎ Corresponding author.
E-mail address: amannhr@hotmail.com (A.A. Abdin).

1056-8727/09/$ – see front matter © 2010 Elsevier Inc. All rights reserved.
doi:10.1016/j.jdiacomp.2009.01.005
the development of diabetic complications (Lobner & Fuchtenbusch, 2004).

Mechanisms, linking inflammation to insulin resistance, are being explored; and progress has been made in this direction (Garg, Tripathy, & Dandona, 2003).

Recently, there has been increasing interest in the active role of adipose tissue in the regulation of metabolism (Pankow et al., 2004).

In this context, adipocytes and macrophages (that is derived from preadipocytes or accumulated directly) are known to secrete inflammatory cytokines [including tumor necrosis factor α (TNF-α), interleukin 6 (IL-6), C-reactive protein (CRP)], as well as free fatty acids (FFAs), most of which have been implicated to play important roles in the cascade of inflammation, systemic insulin resistance, decreased β-cell secretion of insulin, and thus type 2 diabetes (Bastard et al., 2000; Lobner & Fuchtenbusch, 2004; Pankow et al., 2004).

These inflammatory markers have been proposed to be nontraditional risk factors for cardiovascular disease (CVD) in patients with type 2 diabetes mellitus (Haffner et al., 2002).

As a consequence, insulin signaling in adipocytes could become increasingly impaired, eventually leading to massive adipocyte lipolysis, necrosis, and systemic insulin resistance (Xu et al., 2003). Increased lipolysis is one possible piece of the puzzle and could in turn result in the release of a large amount of FFAs, and an increased FFA level in the circulation has been shown to result in resistance to insulin signaling in skeletal muscle and liver (Xu et al., 2003).

Thus, the development of drugs targeted to reverse insulin resistance is an important issue (Yki-Jarvinen, 2004).

The novel class of drugs, thiazolidinediones (e.g., rosiglitazone), which are selective synthetic ligands of the nuclear transcription factor peroxisome proliferator-activated receptor γ (PPAR-γ), act primarily as insulin sensitizers. They are discovered to have anti-inflammatory effects leading to the reevaluation of their potential use in the treatment of diabetes, which may be of considerable clinical significance during long-term therapy (Diamant & Heine, 2003; Garg et al., 2003; Pershadsingh, 2004).

Patients with type 2 diabetes mellitus have a markedly increased risk of cardiovascular complications, where insulin

---

**Table 1**

Values of HOMA-IR and serum levels of TNF-α, IL-6, CRP, FFAs, fasting glucose, and insulin in the studied groups

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Group I n=10</th>
<th>Group II n=7</th>
<th>Group III n=9</th>
<th>Group IV n=8</th>
<th>Group V n=8</th>
<th>Group VI n=10</th>
<th>Group VII n=10</th>
</tr>
</thead>
<tbody>
<tr>
<td>TNF-α (pg/ml)</td>
<td>29.5±2.9</td>
<td>52.7±4.9</td>
<td>45.3±4.2</td>
<td>40.1±3.1</td>
<td>38.7±2.3</td>
<td>34.4±2.4</td>
<td>33.7±2.7</td>
</tr>
<tr>
<td></td>
<td>P&lt;0.01 a</td>
<td>P&lt;0.01 b</td>
<td>NS</td>
<td>P&lt;0.01 b</td>
<td>NS d</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>IL-6 (pg/ml)</td>
<td>37.4±2.3</td>
<td>45.9±3.9</td>
<td>40.3±3.5</td>
<td>39.5±3.1</td>
<td>38.7±2.7</td>
<td>36.9±2.9</td>
<td>36.7±2.5</td>
</tr>
<tr>
<td></td>
<td>P&lt;0.01 a</td>
<td>P&lt;0.05 b</td>
<td>P&lt;0.01 b</td>
<td>P&lt;0.01 b</td>
<td>NS d</td>
<td>NS e</td>
<td>NS</td>
</tr>
<tr>
<td>CRP (mg/dl)</td>
<td>7.9±1.3</td>
<td>12.4±1.9</td>
<td>9.5±1.1</td>
<td>9.1±2.0</td>
<td>8.7±2.3</td>
<td>8.2±1.4</td>
<td>8.1±1.7</td>
</tr>
<tr>
<td></td>
<td>P&lt;0.01 a</td>
<td>P&lt;0.01 a</td>
<td>P&lt;0.01 b</td>
<td>P&lt;0.01 b</td>
<td>NS d</td>
<td>NS e</td>
<td>NS e</td>
</tr>
<tr>
<td>FFAs (mmol/l)</td>
<td>0.35±0.16</td>
<td>0.84±0.28</td>
<td>0.57±0.17</td>
<td>0.49±0.20</td>
<td>0.52±0.19</td>
<td>0.39±0.14</td>
<td>0.40±0.15</td>
</tr>
<tr>
<td></td>
<td>P&lt;0.01 a</td>
<td>P&lt;0.05 b</td>
<td>P&lt;0.05 b</td>
<td>P&lt;0.05 b</td>
<td>NS d</td>
<td>NS e</td>
<td>NS e</td>
</tr>
<tr>
<td>Fasting glucose (mmol/l)</td>
<td>4.84±0.83</td>
<td>8.58±1.35</td>
<td>6.75±1.02</td>
<td>7.05±1.54</td>
<td>7.56±1.16</td>
<td>5.25±0.94</td>
<td>5.48±0.97</td>
</tr>
<tr>
<td></td>
<td>P&lt;0.01 a</td>
<td>P&lt;0.01 a</td>
<td>P&lt;0.01 b</td>
<td>P&lt;0.01 b</td>
<td>NS d</td>
<td>NS e</td>
<td>NS e</td>
</tr>
<tr>
<td>Insulin (μU/ml)</td>
<td>12.13±1.52</td>
<td>14.21±1.71</td>
<td>13.13±1.26</td>
<td>13.82±1.08</td>
<td>13.45±1.51</td>
<td>12.56±1.12</td>
<td>12.48±1.43</td>
</tr>
<tr>
<td></td>
<td>P&lt;0.05 b</td>
<td>NS b</td>
<td>NS b</td>
<td>NS d</td>
<td>NS d</td>
<td>NS e</td>
<td>NS e</td>
</tr>
<tr>
<td>HOMA-IR</td>
<td>2.61±0.15</td>
<td>5.42±0.28</td>
<td>3.94±0.20</td>
<td>4.33±0.19</td>
<td>4.51±0.22</td>
<td>2.93±0.19</td>
<td>3.04±0.21</td>
</tr>
<tr>
<td></td>
<td>P&lt;0.01 a</td>
<td>P&lt;0.01 b</td>
<td>NS d</td>
<td>P&lt;0.01 b</td>
<td>NS</td>
<td>P&lt;0.01 e</td>
<td>P&lt;0.01 e</td>
</tr>
</tbody>
</table>

Values are presented as mean±S.D. P<0.05 (significant); P<0.01 or P<0.001 (highly significant). NS, nonsignificant.

a Group II (diabetic) compared to Group I (control).

b Group III (diabetic with rosiglitazone), Group IV (diabetic with low-dose aspirin), Group V (diabetic with high-dose aspirin), Group VI (diabetic with both rosiglitazone and low-dose aspirin), Group VII (diabetic with both rosiglitazone and high-dose aspirin) compared to Group II (diabetic).

c Group VI (diabetic with both rosiglitazone and low-dose aspirin) and Group VII (diabetic with both rosiglitazone and high-dose aspirin) compared to Group III (diabetic with rosiglitazone).

d Group V (diabetic with high-dose aspirin) compared to Group IV (diabetic with low-dose aspirin).

e Group VII (diabetic with both rosiglitazone and high-dose aspirin) compared to Group VI (diabetic with both rosiglitazone and low-dose aspirin).
resistance is a major determinant of this increased risk and is a potential therapeutic target (Jayagopal, Kilpatrick, Jennings, Hepburn, & Atkin, 2003).

Aspirin, which is currently recommended for patients who have CVD or even CVD risk factors, is shown to ameliorate the diabetic process (Rolka, Fagot-Campagna, Narayan, 2001; Thomas, Nadackal, & Thomas, 2003).

Recent studies demonstrated that salicylates (including aspirin) can reverse hyperglycemia, hyperinsulinemia, and dyslipidemia by sensitizing insulin signaling and improvement of insulin resistance (Jiang, Dallas-Yang, Liu, Moller, & Zhang, 2003; Yin, Yamamoro, & Gaynon, 1998; Yuan et al., 2001).

The cellular and molecular mechanism of the hypoglycemic activity of aspirin has not been well elucidated (Gao, Zuberi, Quon, Dong, & Ye, 2003).

The aim of this work is to study the correlation between insulin resistance and levels of some inflammatory markers (namely, TNF-α, IL-6, CRP) and FFAs in experimental model of induced type 2 diabetes in rats, with evaluation of the effects of rosiglitazone and aspirin (low or high dose), either alone or in combination on the levels of these parameters.

2. Materials and methods

This work was completed on 62 albino rats weighing 150–200 g, served in seven groups as following:

Group I: 10 rats served as normal control group, allowed for ad libitum conventional chow (60% carbohydrate, 22% protein, 8% fiber).

Group II: 7 rats were rendered as model of type 2 diabetes (Zhang et al., 2003), induced experimentally by single intraperitoneal injection of streptozotocin (STZ, Sigma) 15 mg/kg after ad libitum high-fat diet for 2 months (50% carbohydrate, 13% protein, 30% fat, 7% fiber).

This model was designed to mimic the picture of type 2 diabetes in human, where the high-fat diet initiated a state of insulin resistance and then the addition of the relatively low dose of STZ established only a relative insulin deficiency (Zhang et al., 2003).

In Groups III, IV, V, VI, and VII, the rats were rendered diabetic as described before and concomitantly treated by oral gavage as follows:

Group III: nine diabetic rats received rosiglitazone (10 mg/kg, po, daily) (Pickavance, Tadayyon, Widdowson, Buckingham, & Wilding, 1999).

Group IV: eight diabetic rats received low-dose acetyl salicylic acid (10 mg/kg, po, daily) (Patumraj et al., 2000).

Group V: eight diabetic rats received high-dose acetyl salicylic acid (120 mg/kg, po, daily) (Yuan et al., 2001).

Group VI: 10 diabetic rats received both rosiglitazone and low-dose acetyl salicylic acid.

Group VII: 10 diabetic rats received both rosiglitazone and high-dose acetyl salicylic acid.

At the end of the work (2 days after STZ injection), serum samples were obtained to measure levels of TNF-α (pg/ml) and IL-6 (pg/ml) (Zhang, Yu, Huang, Chen, & Wang, 2004); and CRP (mg/ml) (Modzelewski & Janiak, 2004), using the commercially available rat ELISA kits.

FFAs (mmol/l) levels were measured spectrophotometrically by enzymatic colorimetric method, where diphenylcarbazide containing diphenylcarbazone is used as the colored developing reagent (Itaya, 1977).

Because abnormalities in insulin action are poorly detected by a single determination of either glucose or insulin levels (Laakso, 1993 and American Diabetic Association, 1998), the insulin resistance was evaluated by homeostasis model assessment estimate of insulin resistance (HOMA-IR) (Haffner et al., 2002) as follows:

\[ \text{Fasting insulin level (μU/ml)} \times \text{Fasting glucose level (mmol/l)} \]

22.5

Fasting insulin level (μU/ml) was measured using commercial RIA kits (Reaves, 1983), and fasting glucose level (mmol/l) was measured spectrophotometrically by enzymatic colorimetric method (Bdarham & Trinder, 1972).

2.1. Statistics

The values of the measured parameters were presented as mean±S.D. with calculation of the percentage change in their

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Group II(^a) (n=7)</th>
<th>Group III(^b) (n=9)</th>
<th>Group IV(^b) (n=8)</th>
<th>Group V(^b) (n=8)</th>
<th>Group VI(^b) (n=10)</th>
<th>Group VII(^b) (n=10)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TNF-α</td>
<td>↑79%</td>
<td>↑14%</td>
<td>↑24%</td>
<td>↑27%</td>
<td>↑35%</td>
<td>↓36%</td>
</tr>
<tr>
<td>IL-6</td>
<td>↑23%</td>
<td>↑12%</td>
<td>↑14%</td>
<td>↑16%</td>
<td>↑20%</td>
<td>↑20%</td>
</tr>
<tr>
<td>CRP</td>
<td>↑57%</td>
<td>↑23%</td>
<td>↑27%</td>
<td>↑30%</td>
<td>↑34%</td>
<td>↑35%</td>
</tr>
<tr>
<td>FFAs</td>
<td>↑140%</td>
<td>↑32%</td>
<td>↑42%</td>
<td>↑38%</td>
<td>↑54%</td>
<td>↑52%</td>
</tr>
<tr>
<td>HOMA-IR</td>
<td>↑108%</td>
<td>↑27%</td>
<td>↑20%</td>
<td>↑17%</td>
<td>↑46%</td>
<td>↑44%</td>
</tr>
</tbody>
</table>

\(^1\) increase; \(^\downarrow\) decrease.

\(^a\) Percentage change of Group II from Group I (control).

\(^b\) Percentage change of Group II, IV, V, VI, and VII, respectively, from group II.
levels. The difference between each two groups was determined using Student’s t test. The correlation between insulin resistance (HOMA-IR) and each of the inflammatory markers (TNF-α, IL-6, CRP, and FFAs) was evaluated using Pearson correlation coefficient. P values were expressed as P<0.05 (significant), P<0.01, or P<0.001 (highly significant).

All the statistical analyses were processed using Statistical Program of Social Sciences for windows, version 8.0.

3. Results

Table 1 shows values of HOMA-IR and serum levels of TNF-α, IL-6, CRP, FFAs, fasting glucose, and insulin of the all studied groups.

The percentage changes in HOMA-IR and serum levels of TNF-α, IL-6, CRP, and FFAs are shown in Table 2.

The values of the measured parameters of the studied groups are illustrated as follows: HOMA-IR, Fig. 1; TNF-α, Fig. 2; IL-6, Fig. 3; CRP, Fig. 4; and FFAs, Fig. 5.

(1) The results of the present work showed development of insulin resistance in the diabetic group, which is expressed as a significant increase in HOMA-IR when compared to the control group (5.42±0.28 vs. 2.61±0.15; P<0.001).

(2) When compared to the normal control group, the diabetic group showed significant increase in serum levels of TNF-α (52.7±4.9 vs. 29.5±2.9; P<0.001), IL-6 (45.9±3.9 vs. 37.4±2.3; P<0.001), CRP (12.4±1.9 vs. 7.9±1.3; P<0.001), FFAs (0.84±0.28 vs. 0.35±0.16; P<0.001), fasting glucose (8.58±1.35 vs. 4.84±0.83; P<0.001), and insulin (14.21±1.71 vs. 12.13±1.52; P<0.05).

There is significant positive correlation of the studied parameters with HOMA-IR as follows: TNF-α,
(3) The diabetic rats with concomitant administration of rosiglitazone showed significant reduction of the following studied parameters when compared to the diabetic untreated group: HOMA-IR (3.94±0.20 vs. 5.42±0.28, \( P<0.001 \)), TNF-\( \alpha \) (45.3±4.2 vs. 52.7±4.9, \( P<0.01 \)), IL-6 (40.3±3.5 vs. 45.9±3.9, \( P<0.05 \)), CRP (9.5±1.1 vs. 12.4±1.9, \( P<0.01 \)), FFAs (0.57±0.17 vs. 0.84±0.28, \( P<0.05 \)), and fasting glucose (6.75±1.02 vs. 8.58±1.35, \( P<0.01 \)), respectively. However, there is nonsignificant change in insulin level (13.13±1.26 vs. 14.21±1.71).

(4) The diabetic rats administered with low-dose acetyl salicylic acid showed significant reduction of the following studied parameters when compared to the diabetic untreated group: HOMA-IR (4.33±0.19 vs. 5.42±0.28, \( P<0.001 \)), TNF-\( \alpha \) (40.1±3.1 vs. 52.7±4.9, \( P<0.01 \)), IL-6 (39.5±3.1 vs. 45.9±3.9, \( P<0.01 \)), CRP (9.1±2.0 vs. 12.4±1.9, \( P<0.01 \)), FFAs (0.49±0.20 vs. 0.84±0.28, \( P<0.05 \)), and fasting glucose (7.05±1.54 vs. 8.58±1.35, \( P<0.01 \)), respectively. The change in insulin level is nonsignificant (13.82±1.08 vs. 14.21±1.71).

(5) Administration of high-dose acetyl salicylic acid to the diabetic group caused significant reduction of the following studied parameters when compared to the diabetic untreated group: HOMA-IR (4.52±0.22 vs. 5.42±0.28, \( P<0.001 \)), TNF-\( \alpha \) (38.7±2.3 vs. 52.7±4.9, \( P<0.01 \)), IL-6 (38.8±2.9 vs. 45.9±3.9, \( P<0.01 \)), CRP (8.7±2.3 vs. 12.4±1.9, \( P<0.01 \)), FFAs (0.52±0.19 vs. 0.84±0.28, \( P<0.05 \)), and fasting glucose (7.56±1.16 vs. 8.58±1.35, \( P<0.01 \)), respectively. The change in insulin level is nonsignificant (13.45±1.51 vs. 14.21±1.71).

(6) The diabetic rats that received the combination of rosiglitazone with low-dose acetyl salicylic acid showed significant reduction of the following studied parameters when compared to the untreated diabetic group: HOMA-IR (2.93±0.19 vs. 5.42±0.28, \( P<0.001 \)), TNF-\( \alpha \) (34.4±2.4 vs. 52.7±4.9, \( P<0.001 \)), IL-6 (36.9±2.9 vs. 45.9±3.9, \( P<0.001 \)), CRP (8.2±1.4 vs. 12.4±1.9, \( P<0.01 \)), FFAs (0.39±0.14 vs. 0.84±0.28, \( P<0.01 \)), fasting glucose (5.25±0.94 vs. 8.58±1.35, \( P<0.01 \)), and insulin (12.56±1.12 vs. 14.21±1.71, \( P<0.05 \)), respectively.
The diabetic rats that received the combination of rosiglitazone with high-dose acetyl salicylic acid showed significant reduction of the following studied parameters when compared to the untreated diabetic group: HOMA-IR (3.04±0.21 vs. 3.94±0.20, P<0.001), TNF-α (34.4±2.4 vs. 45.3±4.2, P<0.001), IL-6 (36.7±2.5 vs. 45.9±3.9, P<0.001), CRP (8.1±1.7 vs. 12.4±1.9, P<0.001), FFAs (0.41±0.15 vs. 0.84±0.28, P<0.01), fasting glucose (5.48±0.97 vs. 5.85±1.35, P<0.001), and insulin (12.48±1.43 vs. 14.21±1.71, P<0.05), respectively.

When compared to the diabetic rats that received rosiglitazone alone, the diabetic rats that received the combination of rosiglitazone with low-dose acetyl salicylic acid showed significant augmented reduction of the following studied parameters: HOMA-IR (2.93±0.19 vs. 3.94±0.20, P<0.001), TNF-α (34.4±2.4 vs. 45.3±4.2, P<0.001), IL-6 (36.9±2.9 vs. 40.3±3.5, P<0.05), CRP (8.2±1.4 vs. 9.5±1.1, P<0.05), FFAs (0.39±0.14 vs. 0.57±0.17, P<0.05), and fasting glucose (5.25±0.94 vs. 6.75±1.02, P<0.01), respectively. However, there is nonsignificant change in insulin level (12.56±1.12 vs. 13.13±1.26).

Also, when compared to the diabetic rats that received rosiglitazone alone, the diabetic rats that received the combination of rosiglitazone with high-dose acetyl salicylic acid showed significant augmented reduction of the following studied parameters: HOMA-IR (3.04±0.21 vs. 3.94±0.20, P<0.001), TNF-α (33.7±2.7 vs. 45.3±4.2, P<0.001), IL-6 (36.7±2.5 vs. 40.3±3.5, P<0.05), CRP (8.1±1.7 vs. 9.5±1.1, P<0.05), FFAs (0.41±0.15 vs. 0.57±0.17, P<0.05), and fasting glucose (5.48±0.97 vs. 6.75±1.02, P<0.05), respectively. However, there is nonsignificant change in insulin level (12.48±1.43 vs. 13.13±1.26).

There is nonsignificant difference in all studied parameters between the diabetic rats that received high-dose acetyl salicylic acid and those that received low-dose acetyl salicylic acid (HOMA-IR, 4.52±0.22 vs. 4.33±0.19; TNF-α, 38.7±2.3 vs. 40.1±3.1; IL-6, 38.8±2.7 vs. 39.5±3.1; CRP, 8.7±2.3 vs. 9.1±2.0; FFAs, 0.52±0.19 vs. 0.49±0.20; fasting glucose, 7.56±1.16 vs. 7.05±1.54; insulin, 13.45±1.51 vs. 13.82±1.08, respectively).

4. Discussion

According to the new classification and diagnostic criteria for diabetes proposed by the American Diabetes Association, the development of type 2 diabetes is linked to insulin resistance (impaired insulin sensitivity) coupled with a failure of pancreatic cells to compensate by adequate insulin secretion (Fujimoto, 2000 and Gabir et al., 2002).

In the present work, the group of induced type 2 diabetes showed significant increase in serum levels of glucose, insulin, and HOMA-IR value when compared to the normal control group, which indicates development of insulin resistance.

Several studies documented that insulin resistance most often precedes the onset of overt type 2 diabetes and is compensated initially by hyperinsulinemia (Evans et al., 2003 and Zhang et al., 2003). This hyperinsulinemia is produced by both compensatory insulin hypersecretion and by reduced hepatic extraction of insulin (Polonsky et al., 1988). But this chronic secretion of large amounts of insulin to overcome tissue insensitivity can itself finally lead to pancreatic beta cell failure and occurrence of hyperglycemia (Yuan et al., 2001).

It is needed to know the temporal relationship of changes in circulating proinflammatory cytokines, acute-phase markers, insulin resistance, and glycemia during the development of type 2 diabetes. This will raise the question of whether these would be helpful in screening programs identifying individuals at risk of diabetes, and if drugs with anti-inflammatory properties can contribute to the management of the disease (Pickup, 2004).

The results of the present work showed significant increased levels of each of TNF-α, IL-6, and CRP, which are in significant positive correlation with insulin resistance (HOMA-IR) in the diabetic untreated rats.

This is in agreement with the finding of an association between high plasma levels of TNF-α (Nilsson, Jovinge, Neimann, Reneland, & Lithell, 1998) IL-6 (Ridker, Hennekins, Buring, & Rifai, 2000) with insulin resistance and type 2 diabetes.
Also, Leinonen et al. (2003) estimated that all markers of inflammation (including IL-6 and CRP) were positively correlated with HOMA-IR.

Growing evidence has pointed to a correlative and causative relationship between inflammation and insulin resistance/type 2 diabetes mellitus (Xu et al., 2003).

These circulating markers of inflammation (e.g., TNF-α and IL-6) and acute-phase reactants (e.g., CRP) are considered strong predictors of the development of type 2 diabetes and the possible associated cardiovascular complications (Pickup, 2004).

In this context, Temelkova-Kurktschiev et al. (2002) reported that the inflammatory markers were related to insulin resistance but not to insulin secretion.

Recent data have revealed that the plasma concentration of inflammatory mediators, such as TNF-α and IL-6, is increased in the insulin resistant states of obesity and type 2 diabetes. They interfere with insulin action by suppressing insulin signal transduction, and this might interfere with the anti-inflammatory effect of insulin, which in turn might promote inflammation (Dandona, Aljada, & Bandyopadhyay, 2004).

Several researches reported that the circulating TNF-α is usually elevated in established type 2 diabetes (Katsuki et al., 1998; Pickup, Chusney, Thomas, & Burt, 2000; Winkler, Salamon, Speer, Simon, & Cseh, 1998).

Regarding IL-6 and CRP, there are contradictory results obtained by Richardson and Tayek (2002) that their plasma levels did not reach the statistical significant values. However, they explained that this may be due to the small number of subjects and that an ultrasensitive assay is required for accurate measurement of circulating concentrations of IL-6 and CRP.

Also, a controversial result obtained by Bastard et al. (2000) showed that there is nonsignificant correlation between TNF-α and insulin resistance. But this may be attributed to the fact that the correlation was performed collectively for the whole used groups (obese nondiabetic and diabetic) in their study and not to the diabetic group only.

The data presented by Senn, Klover, Nowak, and Mooney (2002) showed that IL-6 plays a direct role in insulin resistance at the cellular level by inhibiting insulin receptor signal transduction and insulin metabolic actions including inhibition of insulin-induced glycogen synthesis.

Regarding TNF-α, it has been reported to inhibit insulin-induced glucose uptake by targeting more than one component of insulin signaling cascade including, insulin receptor, insulin receptor substrate (IRS), and glucose transporter 4. The mechanism of these effects is proved to be due to stimulation of serine phosphorylation of IRS [via activity of the serine kinase inhibitor of nuclear factor-κB (NF-κB) kinase (IKK-β)] leading to both degradation of IRS and inhibition of tyrosine phosphorylation which is essential for insulin signaling and action (Gao et al., 2003).

In addition, the inflammatory cytokines such as TNF-α and IL-6 were reported to down-regulate PPAR-γ expression (Tanaka et al., 1999).

It is known that PPAR-γ is expressed abundantly in adipose tissue, pancreatic beta cells, and macrophages, where it regulates gene transcription of various adipokines, possesses anti-inflammatory activity, and control fatty acid uptake and storage (Yki-Jarvinen, 2004).

In the diabetic group, the elevated FFA level with significant positive correlation with HOMA-IR obtained in this work is in agreement with that estimated in many previous studies (Baldeweg et al., 2000; Laws et al., 1997; Lewis et al., 1991; Reaven, Hollenbeck, Jeng, Wu, & Chen, 1988).

In a study by Pankow et al. (2004), there is no significant association found between FFAs and HOMA-IR. The lack of correlation between increased FFAs and HOMA-IR may be attributed to the fact that FFAs stimulate insulin secretion and some of this insulin is transmitted in the peripheral circulation and is able to compensate for FFA-mediated peripheral insulin resistance (Boden, 1997).

Several mechanisms of how elevated FFA levels decrease insulin sensitivity have been proposed, including the Randle hypothesis concerning inhibition of insulin-stimulated glucose transport. It also should be noted that FFAs regulate gene expression, especially those involved in lipid and carbohydrate metabolism (Evans et al., 2003).

Chronically elevated FFAs may also impair insulin secretory function through toxic effects on pancreatic beta cells as predicted by the “lipotoxicity hypothesis” (Unger, 1995).

Finally, increased flux of FFAs from adipose tissue due to lipolysis of visceral adipose depots (triglycerides) to the nonadipose tissue (e.g., liver, skeletal muscle) may lead to excessive endogenous glucose production and progression to frank type 2 diabetes (Rebrin et al., 1995 and Lewis, Carpentier, Adeli, & Giacca, 2002).

Because insulin resistance both precedes and predicts type 2 diabetes, thus, development of drugs targeted to reverse it is an important issue (Yki-Jarvinen, 2004).

Also, due to link between insulin resistance and inflammatory process, it is suggested that the therapeutic strategies that limit inflammation and reduce levels of inflammatory markers may be a promising tool (Marx et al., 2003).

The insulin sensitizer thiazolidinediones is the first drug to address the basic problem of insulin resistance in type 2 diabetes (Yki-Jarvinen, 2004).

The results of the present work showed that rosiglitazone significantly reduced insulin resistance (HOMA-IR) and glucose level, with nonsignificant decrease in insulin level, which supposed that the improvement in glycemic control is attributed to mechanisms that involve insulin action rather than insulin secretion.

It has been hypothesized that the insulin-sensitizing effects of thiazolidinediones may occur at least in part through decreased lipolysis of adipose tissue and subsequent reduction in circulating FFAs (Mora & Pessin, 2002).

Lewis et al. (2002) reported that PPAR-γ activators (thiazolidinediones), which overcome insulin resistance of adipose tissue by improving adipocyte FFA esterification,
are postulated to be more effective in reducing the deleterious metabolic effects of fat dysregulation.

Recent experimental data suggest that thiazolidinediones (TZDs, glitazones), in addition to their metabolic effects, exhibit anti-inflammatory properties (Marx et al., 2003 and Mohanty et al., 2004).

Yki-Jarvinen (2004) documented that thiazolidinediones exert their insulin-sensitizing actions either (1) directly, by promoting fatty acid uptake and storage in adipose tissue while sparing other insulin-sensitive tissues (mainly skeletal muscle and liver, and possibly pancreatic beta cells) from the harmful metabolic effects of high concentrations of FFAs (the “fatty acid steal” hypothesis), or (2) indirectly, by means of altered adipocytokine release.

The results of the present work showed that rosiglitazone significantly reduced levels of the inflammatory markers and FFAs in the diabetic rats.

Pickup (2004) stated that although the chronic hyperglycemia is not sufficient to induce inflammation, it may contribute to it. So, the improvement in glycemic control may therefore reduce the inflammatory response.

It was established that the decrease in blood glucose level in type 2 diabetes is accompanied with reduced levels of inflammatory markers (Aralich et al., 2000 and Yoo & Desiderio, 2003).

The results of the present work are in agreement with that reported by Miyazaki et al. (2001) that rosiglitazone consistently lowers FFA concentrations in clinical studies. This is confirmed by other recent researches by Goldstein, Cobitz, Hand, and Chen (2003) and Chen et al. (2004) who proved that rosiglitazone significantly reduced FFA level concomitantly with improvement of insulin resistance (HOMA-IR). Meriden (2004) reviewed that rosiglitazone was reported to significantly reduce levels of CRP and FFAs in diabetic cases.

In a study by Haffner et al. (2002), rosiglitazone was shown to improve HOMA-IR and reduce CRP but without significant reduction of IL-6 level. They explained that rosiglitazone treatment has been associated with increase in subcutaneous fat, which is a significant source of IL-6 expression.

In addition, Gee et al. (2004) obtained results of an overall improvement in insulin regulation and lipid profile, although no significant differences were noted in levels of TNF-α and CRP during the treatment period with rosiglitazone. To explain such conflict, Spranger et al. (2003) emphasized that the pathogenesis of type 2 diabetes depends on the combination pattern of inflammatory cytokines rather than on a single cytokine, and they proved that the elevated levels of IL-6 or TNF-α alone are not independently associated with risk of type 2 diabetes.

As reviewed by Nowak and Jaber (2003), the evidences do not support a particular dose of aspirin to be recommended for cardiovascular protection in diabetic patients. The dose of aspirin may be different from that for other populations and requires further evaluation.

For this reason, low and high doses of acetyl salicylic acid were used in the present research to revaluate their effects on glycemic control and inflammatory markers.

The present work showed significant lowering effects on the degree of insulin resistance, levels of the inflammatory markers, and FFAs in the diabetic rats by either low or high dose of aspirin.

Several different clinical studies including in vitro (Gao et al., 2003 and Jiang et al., 2003), in vivo with high doses (6.2 g/day, Hundal et al., 2002; 120 mg/kg per day, Yuan et al., 2001; 1 g t.d.s/day, Mata-Segreda, Fernandez-Azofeifa, Madrigal, & Morales, 1989), and in vivo with low doses (100 mg/day, Contreras et al., 1997) established that acetyl salicylic acid improved insulin resistance with decrease in glucose concentrations and reduction of inflammatory markers including CRP.

The present work showed that administration of aspirin either in low dose or high dose to the diabetic rats resulted in significant lowering of both insulin resistance (HOMA-IR) and glucose level without nonsignificant effect on insulin level, suggesting that aspirin ameliorates glycemic control by insulin-sensitizing effects.

Previously, Giugliano et al. (1985) suggested that the acute insulinotropic effect of acetylsalicylate infusion was due to inhibition of endogenous prostaglandin E (PGE) synthesis, and the concurrent infusion of PGE2 abolished this effect. But this study focused on the acute effect of acetylsalicylate on insulin secretion but not the tissue sensitivity to this insulin on long-term use.

More recent studies (Colwell, 2001; Gao et al., 2003; Hundal et al., 2002; Jiang et al., 2003) proposed several possible mechanisms to mediate the insulin-sensitizing effect of aspirin. First, the molecular events proved the ability of salicylates to inhibit IRS-1 serine phosphorylation, which is strongly implicated in development of insulin resistance, by targeting the serine kinases (e.g., IKK-β). Second, the insulin-sensitizing effect may be attributed to its direct antilipolytic action leading to reduction of the rate of lipolysis and lowering FFA levels. Third, salicylates were proved to decrease insulin clearance and hepatic glucose production. Finally, salicylates were shown to significantly reduce the inflammatory markers (CRP) either by low or high dose, where an anti-inflammatory mechanism was suggested.

Contradictory results were obtained in a previous study by Bratsch-Marrain, Vierhapper, Komjati, and Waldhausl (1985) that showed that administration of acetyl salicylic acid (aspirin) to type 2 diabetic patients (3 g/day for 3 days) caused impairment of tissue sensitivity to insulin during a hyperglycemic clamp study. However, on the other hand, there was decrease in fasting hyperglycemia that was explained by the enhancement of plasma response to insulin.

The results of the present work showed nonsignificant difference between low and high doses of aspirin when administered to the diabetic rats.
Eccles, Freemantle, and Mason (1998) reviewed the guidelines on use of aspirin and reported that the trials have used different doses of aspirin for protection and treatment of CVDs with no evidence that aspirin in doses more than 75 mg daily provides greater benefit.

Although each of rosiglitazone and aspirin is currently prescribed alone or in combination for type 2 diabetes mellitus, but there is no available coherent information about the effect of their combination on insulin resistance, inflammatory markers and FFAs.

In the present work, the groups administered the combination of rosiglitazone and aspirin (either in low or high dose) showed significant decrease in insulin resistance, levels of circulating inflammatory markers, fasting glucose, insulin, and FFAs when compared to the diabetic untreated group.

This significant decrease in insulin level associated with significant improvement of insulin resistance denotes good glycemic control by such combination and is in agreement with the fact that insulin level always reflects changes in insulin sensitivity, where there is compensatory hyperinsulinemia in response to insulin resistance, and vice versa, the insulin level decreased when insulin resistance is improved (Cook & Taborsky, 1996).

When the groups administered the combination of rosiglitazone and aspirin (either in low or high dose) were compared to that administered rosiglitazone alone, there is significant augmented decrease in insulin resistance, levels of circulating inflammatory markers, fasting glucose, and FFAs. These results are in context with the previously discussed results of the present work, which proved that either rosiglitazone or aspirin (at low or high dose) can individually reduce insulin resistance and decrease levels of inflammatory markers and FFAs.

4.1. Conclusion

Insulin resistance forms a hallmark of type 2 diabetes mellitus and is a major determinant of its cardiovascular complications, where the inflammatory pathways play an important role. Therefore, diabetes therapy should focus on this underlying mechanism to provide glycemic control and decrease risk of cardiovascular complications.

Aspirin and rosiglitazone offer unique approaches for the treatment of type 2 diabetes due to their insulin-sensitizing and anti-inflammatory properties. Their combination was found to produce augmented beneficial effects when compared to rosiglitazone alone, providing good glycemic control and consequently decrease risk of cardiovascular complications.

In view of the potential dose-dependent adverse effects of aspirin, with no further benefit achieved by high doses, it is strongly recommended to use low-dose aspirin as a safe and effective medication for diabetes.

In the future, inhibition of the serine kinase IKK-β pathway by aspirin may provide a promising valuable target for discovery of other new drugs for treatment of type 2 diabetes, which needs further researches and evaluation.

References


Giugliano, D., Ceriello, A., Saccamano, F., Quatraro, A., Paolissio, G., & D’Onofrio, F. (1985). Effects of salicylate, tolbutamide, and prostaglan-
din E2 on insulin responses to glucose in noninsulin-dependent diabetes mel-


