Combined Treatment with Interlukin-1 and Tumor Necrosis Factor-Alpha Antagonists Improve Type 2 Diabetes in Rats

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Combined Treatment with Interlukin-1 and Tumor Necrosis Factor-Alpha Antagonists Improve Type 2 Diabetes in Rats

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ABSTRACT

In the present study, combined treatment with etanercept and anakinra were tested in the streptozotocin-induced diabetic rats. Forty male Wistar albino rats were divided into 5 groups; healthy control (HC), diabetic control (DC), diabetic+anakinra (DAT), diabetic+etanercept (DET), and diabetic+etanercept+anakinra (DEAT). HC and DC groups received subcutaneous (sc.) injection with a saline solution, while DAT and DET groups received anakinra (10 mg/kg/day, sc.) or etanercept (10 mg/kg, twice a week, sc.), and DEAT rats received both anakinra and etanercept treatments for 21 days after diabetes has developed. Anakinra and etanercept treatments significantly increased insulin and homeostatic model assessment-β cell function levels and decreased glucose levels compared to the DC group as single (DAT and DET) and combined treatments (DEAT). The thiobarbituric acid reactive substances level was significantly decreased in DAT group. The combine use of etanercept and anakinra can improve insulin and blood glucose in type 2 diabetic rats. The combined treatment of anakinra and etanercept together was more effective than single treatment and might have a potential new treatment strategy and to reduce the mortality and morbidity resulting from diabetes.

Keywords: Etanercept; anakinra; type 2-diabetes mellitus; interleukin-1; tumor necrosis factor-alpha.

1. Introduction

Type 2 diabetes has now become one a serious worldwide public health problem (Selvaraju et al. 2012). Diabetes is characterized by reduced insulin secretion or defects in insulin activity resulting in consistently high levels of blood glucose and requiring chronic treatment (Bas et al. 2012). High blood insulin levels are found in the early stages of the disease due to increased resistance to insulin actions, which then develops into insulin dependent diabetes as the disease progresses. Hyperglycemia develops due to disrupted
secretion and increased insulin resistance results in glucotoxicity (Bas et al. 2012; Del Prato 2009; Maris et al. 2010).

Insulin resistance is a decreased physiological response to the normal effect of insulin. There is a growing consensus in the literature that inflammation plays an important role in mediating glucotoxicity, insulin resistance, and other mechanisms underlying diabetes (Wellen and Hotamisligil 2003). Chronically high glucose concentration results in coronary, cerebrovascular and peripheral vascular diseases, nephropathy, neuropathy, and retinopathy in long-term. For this purpose, the blood glucose level should be kept within normal limits (Quinn 2002; Sailaja et al. 2003).

Endoplasmic reticulum stress and activation of inflammatory pathways in diabetes mellitus, infiltration of inflammatory mediators in tissues (adipose tissue, pancreas and other metabolic tissues) and circulation, are the main causes of inflammation (Herder et al. 2013). Increased tumor necrosis factor alpha (TNF-α) and interleukin-1 beta (IL-1β) levels play a major role in this chronic inflammation (Pan et al. 2010; Selvaraju et al. 2012). High blood glucose and elevated blood lipid levels in type 2 diabetes have been reported to result in chronic inflammation (Das and Mukhopadhyay 2011; Goldfine et al. 2011). Inflammation and inflammatory markers (TNF-α, IL-1, IL-6, etc.) are also an important risk factor in the disease (Das and Mukhopadhyay 2011).

Adipocytes have an important role in the development and progression of diabetes through the secretion of peptide hormones and other biologically active molecules such as cytokines (TNF-α, IL-6, IL-1), angiotensinogen, plasminogen activator inhibitor-1, leptin and adiponectin (Coelho et al. 2013). The excessive secretion of cytokines such as TNF-α and IL-6 leads to chronic inflammation and can result insulin resistance and hyperglycemia promoting the development of type 2 diabetes (Coelho et al. 2013; Das and Mukhopadhyay 2011).
The dysfunction in β cells, the insulin secreting cells of the pancreas, is an important factor in the development of diabetes and can be promoted by diabetic hyperglycemia, macrophage infiltration, increased proinflammatory cytokines, and oxidative stress decreasing the capacity to secrete insulin (Akash et al. 2013). Depending on these factors, amyloid polypeptides are produced from the islets and these polypeptides interact with the inflammatory cells and cause inflammation in the pancreatic β islets (Donath and Halban 2004).

High glucose and free fatty acid levels leads to increase activity of IL-1β, activation of nuclear factor kappa (NF-kB) pathway and develop endoplasmic reticulum stress, mononuclear cell infiltration and activation, and insulin-dependent disorders. After these events, expression of IL-1β, TNF-α which are proinflammatory cytokine and interferon gamma (IFN-γ) increase and apoptosis occur pancreatic β-cells. This chronic cycle leads to chronic inflammatory and organ damage, insulin resistance and insulin dependent patients (Akash et al. 2013; Cnop et al. 2005).

The treatment with IL-1 antagonists has been reported to reduce hyperglycemia, dysfunction of β cells, and systemic inflammation in type 2 diabetes (Donath and Halban 2004; Larsen et al. 2007). In addition, it enhances β cell secretory capacity and induces insulin (Herder et al. 2013; Larsen et al. 2007) and improve HbA1c levels. This has been explained by the chronic inflammation depending on Jun-kinase (JNK) and NF-κB activation and the increase in levels of TNF-α and IL-1β (Herder et al. 2013). Anakinra that is IL-1 antagonist, have reported decreased inflammation in tissues, increased insulin sensitivity, and improved β-cell function in diabetic rats (Donath and Shoelson 2011; Ehses et al. 2009).

The use of TNF-α antagonists in patients with type 2 diabetes has been suggested to be important in preventing systemic inflammation and enhancing β-cell function (Dominguez et al. 2005). However, evidence has indicated that etanercept treatment may result in
hypoglycemia (Bonilla et al. 2007). Etanercept treatment in individuals with metabolic syndrome reduces inflammation lowering glucose to normal levels, insulin resistance (Stanley et al. 2011).

As a result, the development of IL-1 and TNF-α receptor antagonists have been a new target in the treatment of type 2 diabetes in recent years (Ehses et al. 2009; Stanley et al. 2011). The combined usage of etanercept and anakinra may reduce the chronic inflammation, hyperglycemia, β-cell dysfunction, and insulin resistance occurred in type 2 diabetic rats (Dominguez et al. 2005; Donath and Halban 2004; Larsen et al. 2007). In this study, we aimed the determination of endocrinological, biochemical, hematologic, oxidative stress and antioxidant status following single or combine administration of etanercept and anakinra in experimentally induced type 2 diabetic rats.

2. Materials and Methods

2.1. Animals

A total of forty Wistar Albino rats of similar weights (200-250 g) for 6-10 weeks were used. Health checks were conducted before the rats were included in the study. Pellet rat diet (Bil-Yem Feeds, Ankara, Turkey) and water were provided ad libitum throughout the study. The composition of diet given to healthy control (HC) group consisted of; dry matter: 89%, hp: 21%, cellulose: up to 5%, ash: up to 10%, Ca: 1-2%, P: 0.5-1%, NaCl: 0.5% 2850 kcal/kg. Rats were housed in standard polypropylene cages in a controlled environment with automatic ambient humidity 55 ± 5%, a temperature of 22 ± 2°C, and a daily 12-h light/12 h dark cycle. The animals were obtained from the Selcuk University Chair of Experimental Medicine Research and Application Center (SUCEMRAC), Konya, Turkey. Research protocol was reviewed and approved by the Ethic Committee of SUCEMRAC (the decision dated 27.10.2015 and numbered 2015/92). All experiments and manipulations were carried out in accordance with the Guide for the Care and Use of Laboratory Animals (1996, published by

2.2. Development of Experimental Type 2 Diabetes Model and Experimental Design

An experimental model of type 2 diabetes was induced in the rats following the method detailed by Srinivasan (Srinivasan et al. 2005). Rats were fed by high fat diet (HFD) containing 58% of total kcal as fat ad libitum for 2 weeks. HFD composition included 3% vegetable oil, 37% animal fat as tallow, 30.5% corn, 20% casein, 4.5% soy pulp, 1.7% dicalcium phosphate, 0.2% dl-methionine, 1.6% lime stone, 0.5% salt, and a vitamin-mineral blend 1%. Streptozotocin (STZ, ≥98% [HPLC], Sigma-Aldrich Co., USA) was dissolved in citrate buffer (pH 4.5, 20 mg/mL). After 2 weeks of HFD feeding, a subset of the rats was injected intraperitoneally (i.p.) with low dose STZ (35 mg/kg). Seven days after STZ injection, rats with the non-fasting glucose level of ≥300 mg/dl were considered as diabetic. Rats were fed on HFD ad libitum until the end of the study. They were grouped as follows:

1. Group (HC) [Healthy control (n = 6)]: The animals in this group were given saline (0.1 ml / rat / day, s.c.) for 21 days;
2. Group (DC) [Diabetic Control (n = 8)]: Saline (0.1 ml/rat/day, s.c.) were given in experimental diabetic 8 rats for 21 days;
3. Group (DAT) [Diabetic + Anakinra Treatment (n = 8)]: Anakinra (10 mg/kg/day, s.c.) (Ehses et al. 2009) as an IL-1 antagonist, was administered in experimental diabetic 8 rats for 21 days;
4. Group (DET) [Diabetic + Etanercept Treatment (n = 8)]: TNF-α antagonist etanercept (10 mg/kg, twice weekly s.c.) (Dogrul et al. 2011) was administered in experimental diabetic 8 rats for 21 days;
5. Group (DEAT) [Diabetic + Etanercept and Anakinra Treatment (n = 10)]: Experimental diabetic 10 rats were given a combination therapy of etanercept (10 mg/kg, s.c. twice a week) and anakinra (10 mg/kg/day, s.c.) for 21 days.

At the end of the 21 days, blood samples were collected from the retro-orbital sinus of animals under thiopental Na anesthesia (40 mg/kg, i.p.) and rats were then euthanized by cervical dislocation.

2.3. Hematological, biochemical, endocrinological, oxidative status, and antioxidant capacity analysis

Hemogram parameters (erythrocyte, leukocyte, platelet, hematocrit, and hemoglobin) were analyzed using a blood cell counter (BC-2800 Auto Hematology Analyzer, Mindray Bio-Medical Electronics, Shenzen, China) with blood samples collected in anticoagulant tubes (K2 EDTA). To generate serum samples blood was centrifuged at 1600 g and the serum separated. Biochemical analysis (albumin, alanine aminotransferase: ALT, aspartate aminotransferase: AST, creatine kinase: CK, creatine kinase muscle/brain: CK-MB, hemoglobin A1c: HbA1c, total bilirubin: T-BIL, glucose, urea, creatinine, low density lipoprotein: LDL, high density lipoprotein: HDL, cholesterol, triglyceride: TRIG, total protein) were measured from serum and whole blood with autoanalyzer (Cobas Integra 400 plus, Roche Diagnostics Ltd., Rotkreuz, Switzerland) using a commercial autoanalyzer kit (Cobas Integra 400 plus assay kit).

Insulin (Ultra-Sensitive Rat Insulin ELISA Kit, Catalog No. 90060, Crystal Chem, USA), adiponectin (Rat ADP/Acrp 30 ELISA Kit, Catalog No. E-EL-R0329, Elabscience Biotechnology Co. Ltd., China), leptin (Rat LEP ELISA Kit, Catalog No. R0582, Elabscience Biotechnology Co. Ltd., China), resistin (Rat RTN ELISA Kit, Catalog no: E-EL-R0614, Elabscience Biotechnology Co. Ltd., China) and total oxidative stress indicator Thiobarbituric Acid Reactive Substances (TBARS Assay Kit, Item no: 1009055, Cayman Chemical...
Company, USA), total antioxidant capacity indicator (TAC, Antioxidant Assay Kit, Item no: 709001, Cayman Chemical Company, USA) were assayed by ELISA reader (Bio-Tek Instruments Inc., MWGt Lambda Scan 200) following to manufacturer’s protocol.

Insulin sensitivity tests (Homeostatic Model Assessment-Insulin Resistant: HOMA-IR and Homeostatic Model Assessment-β cell function: HOMA-β) were calculated with the determined glucose and insulin values according to the following formula:

HOMA-IR: \( \frac{\text{Glucose} \times \text{Insulin}}{405} \),

HOMA-β: \( \frac{360 \times \text{Insulin}}{\text{Glucose} - 63} \).

2.5. Statistical Analysis

The distribution of values were tested for normality using SPSS 22.0 (SPSS, Inc., Chicago, IL, USA). Statistical significance among groups was tested using the Kruskal-Wallis and post hoc Mann-Whitney U test for parameters (diabetic and endocrinological parameters) that were not normally distributed and using one-way ANOVA for normally distributed parameters (other parameters) (SPSS 22.0) and posthoc Scheffe test. In all cases, \( p < 0.05 \) was considered statistically significant.

3. Results

The changes in diabetic parameters are presented in Table 1 after anakinra and etanercept treatment in experimental type 2 diabetic rats. Insulin was higher (\( p < 0.05 \)) in DAT, DET, and DEAT groups than DC. DET and DEAT groups were similar to the HC group. Blood glucose level was significantly highest in the DC group when compared with the DAT, DET, and DEAT groups (\( p < 0.05 \)). HbA1c level was higher (\( p < 0.05 \)) in the DC group than HC group. HOMA-IR level were lower (\( p < 0.05 \)) in the DEAT group than DC group.
while HOMA-IR value of DEAT group was similar to HC group (Table 1). HOMA-β value was higher (p < 0.05) in the treatment groups (DAT, DET and DEAT) than the DC group and the DC group was different from the HC group (Table 1).

Adiponectin, resistin, and leptin levels remained unchanged between the experimental groups (Table 2).

Biochemical results of the study were variable (Table 3). HDL was lowest in the DC group, while HDL levels were higher (p < 0.05) in the DET and DEAT. In addition, HDL level in DAET was similar in the HC group. Liver enzymes ALT and AST were lower (p < 0.05) in the DC group compared to the HC group. The cardiac injury marker CK-MB level was lower (p < 0.05) in the DEAT group than the HC and DC groups (Table 3). RBC and hematocrit levels were higher (p < 0.05) in the DEAT group compared to the other groups (Table 4).

TAC was unchanged between the different groups (Fig 1). In contrast, TBARS was lowest (p < 0.05) in DAT group compared to the DC group while the DET and DEAT groups were non-significantly lower than the DC group (Fig 2).

4. Discussion

In the study, we confirmed that insulin secretion and HOMA-β levels decreased and HOMA-IR level increased in the DC group due to impaired β-cell function. While the decreased insulin levels were prevented by the anakinra treatment, etanercept was completely treated in DET and DEAT groups and the insulin levels was observed the same level with HC group. The HOMA-β levels were partially treated by anakinra or etanercept treatments alone, while the combination of both anakinra and etanercept (DEAT) restored HOMA-β to the same level as in the HC group (Table 1). The single use of etanercept and combine usage with anakinra has been previously shown to suppress TNF-α and IL-1 in a study of experimental transplantation of pancreatic β islets in mice, improving insulin secretion and metabolic status
of the grafted cells (McCall et al. 2012). Increased TNF-α is directly toxic for β cells both \textit{in vivo} (Kägi et al. 1999) and \textit{in vitro} (Zhang and Kim 1995). In diabetic patients, the administration of etanercept at a dose of 25 mg/kg (twice a week) for 4 weeks was effective in suppressing inflammation, improving β-cell insulin secretion and increasing HOMA-β levels in the acute phase without altering insulin sensitivity in the short term (Dominguez et al. 2005). However, the pharmacological inhibition of TNF-α and NF-κB has been shown to reduce insulin resistance (Yang et al. 2009). Increased IL-1β levels in diabetes can also lead to β cell dysfunction, apoptosis, decreased insulin secretion, insulin resistance, oxidative stress, and mitochondrial dysfunction (van Asseldonk et al. 2010; Vitale et al. 2015). Antagonism of IL-1 via anakinra administration improves hyperglycemia and β-cell function (Larsen et al. 2007). This effect could be explained by IL-1 antagonists directly inhibit IL-1 effects and through indirect suppression of other cytokines such as IL-6 and TNF-α in β-cells (Ehses et al. 2009). In the current study, it was observed that the level of insulin in etanercept treatment (DET and DEAT) groups was similar to HC group and indicating that the inhibition of TNF-α signaling can prevent the decreased levels of insulin in diabetics. However, combined usage of anakinra and etanercept can be more effective in the treatment of diabetes due to the inhibition of both TNF-α and IL-1β. In addition, this combined treatment may have improved the function of β cells and insulin secretion and reduced insulin resistance.

In the study, glucose was significantly higher in the DC group than the other experimental groups (DAT, DET, DEAT, HC groups) and it statistically decreased in DAT, DET, DEAT groups (Table 1). Unlike HbA1c levels were not change in the treatment groups than the HC group (Table 1). Anakinra treatment in patients with type 2 diabetes can improve hyperglycemia by increasing insulin secretion capacity of β cells resulting in decreased HbA1c levels with these beneficial effects increasing with longer duration of treatment (Ehses et al. 2009; Larsen et al. 2007). Etanercept treatment suppresses TNF-α effects, reduces
inflammation and glucose level by increasing insulin sensitivity of cells in metabolic syndrome patients (Stanley et al. 2011). High-dose aspirin treatment in type 2 diabetic patients decreases insulin clearance, reduces glucose levels, and increases insulin sensitivity by suppressing Iκ-B, an important component in inflammation pathways (Hundal et al. 2002). In the current study, suppression of TNF-α and IL-1 in the IκB pathway in treatment groups (DAT, DET, DEAT groups) can provide an explanation for the reduced levels of endogenous glucose and this decrease was more prominent in the combined anakinra and etanercept treatment group.

IL-1 inhibition with anakinra can cause different effects on insulin and glucose levels, depending on the dosage (Ehses et al. 2009; Lacraz et al. 2009). The use of TNF-α inhibitors in type 2 diabetes may increase insulin secretion or insulin sensitivity, although this effect has been questioned (Dominguez et al. 2005; Martinez-Abundis et al. 2007). The current study data indicates that TNF-α inhibition can reduce the level of glucose by increasing β-cell function through the prevention of inflammation.

In the current study, some biochemical parameters such as ALT, AST and CK-MB were statistically altered among the groups. However, these differing parameters remained within the reported normal reference range for rats (Petterino and Argentino-Storino 2006; Tasgin et al. 2017).

The lack of changes in endocrinological and some biochemical (TRIG, CHOL, LDL, AST, ALT, CK, T-BIL, ALB, T-PROT, CK-MB, UREA, CREA) parameters in the present study may have been due to the short duration of the study as the experimental diabetic rat model used lasted less than three months (Larsen et al. 2007; Stanley et al. 2011).

HDL levels were statistically increased in the etanercept treated groups (DET, DEAT groups) compared to DC group. Etanercept treatment decreases lipid accumulation in the liver and adipose tissues and can result in hypoglycemia in diabetic patients (Bonilla et al. 2007).
In the current study etanercept treatment may have prevented lipid accumulation due to the to increased level of HDL.

The RBC and hematocrit parameters were statistically different among the groups but remained within the normal limits for the rats (Petterino and Argentino-Storino 2006). However, decrease of RBC level can be explained as the blocking circulation of RBC due to increased bone marrow suppression of cytokines such as IL-1 and TNF-α (Lorenzo 2000).

The TAC values were not significantly changed but a small decrease was noted in the DC group. TBARS was significantly lower in the DAT group than in the DC group. It has been reported that hyperglycemia reduces antioxidant activity by suppressing glutathione reductase and superoxide dismutase enzymes. At the same time, glycation products trigger oxidative stress. MDA level increases, and TAC level decreases due to lipid peroxidation in rats fed high fat diet (West 2000; Yang et al. 2009). The suppression of proinflammatory cytokines such as TNF-α and IL-1 reduce oxidative stress (Wahl et al. 1998). Hyperglycemia may damage and disrupts function of β cells via causing oxidative stress (Donath and Shoelson 2011; Gorogawa et al. 2002). Treatment with etanercept and anakinra suppressed TNF-α and IL-1, reducing both inflammation and hyperglycemia so potentially reducing reactive oxygen species and this may have partially reduced the TBARS level and increased the TAC level. However, lack of statistical differences in TBARS and TAC levels of treatment groups may be due to the short duration of the experiment.

In conclusion, treatment with etanercept and anakinra increases insulin and HOMA-β levels, while reducing glucose, HOMA-IR, and TBARS levels in this rat model of type 2 diabetes. These results indicate that anti-inflammatory therapy may have treated the insulin sensitivity and lowered the glucose level by inhibiting the inflammatory mechanism specifically at the cytokine level. As a result, the combined inhibition of both TNF-α and IL-1 is more effective than either treatment alone and the combination of etanercept and anakinra
Draft is a promising potentially treatment for diabetes.

Acknowledgments

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References


Table 1. Effect of single and combine therapy of anakinra (10 mg / kg / day, SC) and etanercept (10 mg / kg, twice weekly, SC) on diabetic parameters in experimental type 2 diabetic rats (mean ± SE).

<table>
<thead>
<tr>
<th>Parameters</th>
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<th>DC</th>
<th>DAT</th>
<th>DET</th>
<th>DEAT</th>
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<tr>
<td>Insulin (ng/mL)</td>
<td>4.46 ± 0.47&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.05 ± 0.29&lt;sup&gt;c&lt;/sup&gt;</td>
<td>2.67 ± 0.26&lt;sup&gt;b&lt;/sup&gt;</td>
<td>3.13 ± 0.39&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.45 ± 0.29&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Glucose(mg/dL)</td>
<td>147.8 ± 9.9&lt;sup&gt;c&lt;/sup&gt;</td>
<td>399.7 ± 48.9&lt;sup&gt;a&lt;/sup&gt;</td>
<td>251.6 ± 50.6&lt;sup&gt;b&lt;/sup&gt;</td>
<td>250.6 ± 47.5&lt;sup&gt;b&lt;/sup&gt;</td>
<td>178.0 ± 16.68&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>HbA1c (%)</td>
<td>3.8 ± 0.04&lt;sup&gt;b&lt;/sup&gt;</td>
<td>6.47 ± 0.66&lt;sup&gt;a&lt;/sup&gt;</td>
<td>6.08 ± 0.58&lt;sup&gt;a&lt;/sup&gt;</td>
<td>5.76 ± 0.62&lt;sup&gt;a&lt;/sup&gt;</td>
<td>5.68 ± 0.39&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>HOMA-IR</td>
<td>38.0 ± 3.24&lt;sup&gt;b&lt;/sup&gt;</td>
<td>47.1 ± 2.34&lt;sup&gt;a&lt;/sup&gt;</td>
<td>41.7 ± 6.68&lt;sup&gt;a&lt;/sup&gt;</td>
<td>45.7 ± 5.85&lt;sup&gt;a&lt;/sup&gt;</td>
<td>37.7 ± 3.29&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>HOMA-β</td>
<td>506.0 ± 108.5&lt;sup&gt;a&lt;/sup&gt;</td>
<td>54.0 ± 18.4&lt;sup&gt;c&lt;/sup&gt;</td>
<td>201.9 ± 74.1&lt;sup&gt;b&lt;/sup&gt;</td>
<td>187.4 ± 36.4&lt;sup&gt;b&lt;/sup&gt;</td>
<td>304.6 ± 57.4&lt;sup&gt;b&lt;/sup&gt;</td>
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</table>

HC: Healthy Control, DC: Diabetic Control, DAT: Diabetic+Anakinra Treatment, DET: Diabetic+Etanercept Treatment, DEAT: Diabetic+Etanercept and Anakinra Treatment, HOMA-IR: Homeostatic Model Assessment - Insulin Resistant, HOMA-β: Homeostatic Model Assessment – β cell function, HbA1c (%): Hemoglobin A1c, <sup>a,b,c</sup>: Different letters in the same line are statistically significant (P<0.05).
Table 2. Effect of single and combine therapy of anakinra (10 mg / kg / day, SC) and etanercept (10 mg / kg, twice weekly, SC) on endocrinological parameters in experimental type 2 diabetic rats (mean ± SE).

<table>
<thead>
<tr>
<th>Parameters</th>
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<th>DAT</th>
<th>DET</th>
<th>DEAT</th>
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<tbody>
<tr>
<td>ADP(Acrp30) (pg/mL)</td>
<td>348.8 ± 52.0a</td>
<td>311.0 ± 58.8a</td>
<td>362.2 ± 107.0a</td>
<td>350.8 ± 53.2a</td>
<td>432.8 ± 97.1a</td>
</tr>
<tr>
<td>Resistin (ng/mL)</td>
<td>0.37 ± 0.25a</td>
<td>2.88 ± 2.3a</td>
<td>0.52 ± 0.12a</td>
<td>0.23 ± 0.16a</td>
<td>0.45 ± 0.09a</td>
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<tr>
<td>Leptin (ng/mL)</td>
<td>0.13 ± 0.07a</td>
<td>1.36 ± 1.06a</td>
<td>0.19 ± 0.12a</td>
<td>0.12 ± 0.07a</td>
<td>0.56 ± 0.36a</td>
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ADP(Acrp30): Adiponectin, a: There is no statistical difference between the groups (P> 0.05). (P<0.05).
Table 3. Effect of single and combine therapy of anakinra (10 mg / kg / day, SC) and etanercept (10 mg / kg, twice weekly, SC) on biochemical parameters in experimental type 2 diabetic rats (mean ± SE).

<table>
<thead>
<tr>
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<tr>
<td>TRIG (mg / dL)</td>
<td>95.5 ± 10.09\textsuperscript{a}</td>
<td>215 ± 22.71\textsuperscript{a}</td>
<td>179.4 ± 43.51\textsuperscript{a}</td>
<td>150.25 ± 31.37\textsuperscript{a}</td>
<td>149.2 ± 22.34\textsuperscript{a}</td>
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<td>CHOL (mg / dL)</td>
<td>74.0 ± 4.2\textsuperscript{a}</td>
<td>71.25 ± 5.9\textsuperscript{a}</td>
<td>69.6 ± 5.02\textsuperscript{a}</td>
<td>74.8 ± 4.22\textsuperscript{a}</td>
<td>77.4 ± 1.69\textsuperscript{a}</td>
</tr>
<tr>
<td>HDL (mg/dL)</td>
<td>56.0 ± 2.13\textsuperscript{a}</td>
<td>33.0 ± 2.12\textsuperscript{a}</td>
<td>42.2 ± 2.35\textsuperscript{bc}</td>
<td>46.4 ± 2.78\textsuperscript{ab}</td>
<td>54.0 ± 1.82\textsuperscript{a}</td>
</tr>
<tr>
<td>LDL (mg/dL)</td>
<td>11.40 ± 0.51\textsuperscript{a}</td>
<td>12.0 ± 1.22\textsuperscript{a}</td>
<td>11.4 ± 1.08\textsuperscript{a}</td>
<td>11.0 ± 1.08\textsuperscript{a}</td>
<td>12.2 ± 0.7\textsuperscript{a}</td>
</tr>
<tr>
<td>AST (U/L)</td>
<td>135.5 ± 7.12\textsuperscript{a}</td>
<td>97.6 ± 14.54\textsuperscript{ab}</td>
<td>83.3 ± 12.18\textsuperscript{b}</td>
<td>73.8 ± 4.38\textsuperscript{b}</td>
<td>63.3 ± 6.33\textsuperscript{b}</td>
</tr>
<tr>
<td>ALT (U/L)</td>
<td>48.00 ± 2.74\textsuperscript{a}</td>
<td>33.20 ± 2.01\textsuperscript{b}</td>
<td>33.5 ± 3.38\textsuperscript{b}</td>
<td>29.0 ± 1.73\textsuperscript{b}</td>
<td>31.2 ± 2.65\textsuperscript{b}</td>
</tr>
<tr>
<td>CK (U/L)</td>
<td>990.2 ± 82.23\textsuperscript{a}</td>
<td>822.4 ± 133.84\textsuperscript{a}</td>
<td>623.8 ± 80.42\textsuperscript{a}</td>
<td>714.4 ± 128.9\textsuperscript{a}</td>
<td>547.6 ± 129.9\textsuperscript{a}</td>
</tr>
<tr>
<td>T-BİL (mg/dL)</td>
<td>0.18 ± 0.01\textsuperscript{a}</td>
<td>0.19 ± 0.05\textsuperscript{a}</td>
<td>0.22 ± 0.05\textsuperscript{a}</td>
<td>0.18 ± 0.02\textsuperscript{a}</td>
<td>0.17 ± 0.01\textsuperscript{a}</td>
</tr>
<tr>
<td>ALB (g/dL)</td>
<td>3.22 ± 0.06\textsuperscript{a}</td>
<td>3.17 ± 0.09\textsuperscript{a}</td>
<td>2.90 ± 0.18\textsuperscript{a}</td>
<td>3.11 ± 0.08\textsuperscript{a}</td>
<td>3.12 ± 0.12\textsuperscript{a}</td>
</tr>
<tr>
<td>T-PROT (g/dL)</td>
<td>6.66 ± 0.11\textsuperscript{a}</td>
<td>6.00 ± 0.74\textsuperscript{a}</td>
<td>6.81 ± 0.28\textsuperscript{a}</td>
<td>7.28 ± 0.34\textsuperscript{a}</td>
<td>7.32 ± 0.19\textsuperscript{a}</td>
</tr>
<tr>
<td>CK-MB (U/L)</td>
<td>1000 ± 0.0\textsuperscript{a}</td>
<td>958.2 ± 41.8\textsuperscript{a}</td>
<td>952.3 ± 47.7\textsuperscript{a}</td>
<td>796.0 ± 73.8\textsuperscript{a}</td>
<td>586.9 ± 82.3\textsuperscript{b}</td>
</tr>
<tr>
<td>UREA (mg/dL)</td>
<td>44.33 ± 0.88\textsuperscript{a}</td>
<td>45.17 ± 2.56\textsuperscript{a}</td>
<td>48.25 ± 3.16\textsuperscript{a}</td>
<td>44.71 ± 2.85\textsuperscript{a}</td>
<td>37.89 ± 2.41\textsuperscript{a}</td>
</tr>
<tr>
<td>CREA (mg/dL)</td>
<td>0.58 ± 0.02\textsuperscript{a}</td>
<td>0.6 ± 0.02\textsuperscript{a}</td>
<td>0.57 ± 0.03\textsuperscript{a}</td>
<td>0.58 ± 0.02\textsuperscript{a}</td>
<td>0.64 ± 0.02\textsuperscript{a}</td>
</tr>
</tbody>
</table>

TRIG: Triglyceride, CHOL: Cholesterol, HDL: High-Density Cholesterol, LDL: Low Density Cholesterol, AST: Aspartate Aminotransferase, ALT: Alanine Aminotransferase, CK: Creatine Kinase, T-BİL: Total Bilirubin, ALB: Albumin, T-PROT: Total Protein, Protein, CK-MB: Creatine kinase-MB, CREA: Creatinine, a, b, c: Different letters in the same line are statistically significant (P<0.005).
Table 4. Effect of single and combine therapy of anakinra (10 mg / kg / day, SC) and etanercept (10 mg / kg, twice weekly, SC) on haemotological parameters in experimental type 2 diabetic rats (mean ± SE).

<table>
<thead>
<tr>
<th>Parameters</th>
<th>HC</th>
<th>DC</th>
<th>DAT</th>
<th>DET</th>
<th>DEAT</th>
</tr>
</thead>
<tbody>
<tr>
<td>WBC (x10^9/L)</td>
<td>10.3 ± 0.71^a</td>
<td>6.3 ± 0.53^a</td>
<td>6.65 ± 0.97^a</td>
<td>5.58 ± 0.45^a</td>
<td>9.54 ± 1.57^a</td>
</tr>
<tr>
<td>RBC (x10^12/L)</td>
<td>8.49 ± 0.17^ab</td>
<td>8.06 ± 0.12^b</td>
<td>8.14 ± 0.16^b</td>
<td>7.93 ± 0.13^b</td>
<td>8.94 ± 0.19^a</td>
</tr>
<tr>
<td>Platelet (x10^9/L)</td>
<td>317.2 ± 40.8^a</td>
<td>177.2 ± 61.1^a</td>
<td>272.7 ± 38.4^a</td>
<td>350.7 ± 38.3^a</td>
<td>336.9 ± 27.8^a</td>
</tr>
<tr>
<td>Hemoglobin (g/dL)</td>
<td>15.23 ± 0.23^a</td>
<td>15.48 ± 0.77^a</td>
<td>14.67 ± 0.38^a</td>
<td>14.47 ± 0.37^a</td>
<td>16.44 ± 0.4^a</td>
</tr>
<tr>
<td>Hematocrit %</td>
<td>46.9 ± 0.91^dh</td>
<td>43.9 ± 0.58^b</td>
<td>44.81 ± 1.31^b</td>
<td>44.13 ± 0.53^b</td>
<td>51.08 ± 1.35^a</td>
</tr>
</tbody>
</table>

WBC: White blood cell, RBC: Red blood cell, ^a, b: Different letters in the same line are statistically significant (P<0.05).
Figure 1. Effect of single and combine therapy of anakinra (10 mg / kg / day, SC) and etanercept (10 mg / kg, twice weekly, SC) on Total Antioxidant Capacity (TAC) in experimental type 2 diabetic rats (mean).
Figure 2. Effect of single and combine therapy of anakinra (10 mg / kg / day, SC) and etanercept (10 mg / kg, twice weekly, SC) on Thiobarbituric Acid Reactive Substances (TBARS) in experimental type 2 diabetic rats (mean).