EFFECT OF ETANERCEPT AND DIACEREIN IN HIGH FRUCTOSE-INDUCED METABOLIC SYNDROME

By
Walaa Yehia Abdel-Zaher, Mohamed Abdellah Ibrahim, Salwa Abdel-tawab Ibrahim, Entesar Farghaly Amin and Aly Mohamed Abdelrahman
Department of Pharmacology, El-Minia Faculty of medicine

ABSTRACT:

Metabolic syndrome (MS) is a complex of medical abnormalities namely obesity, dyslipidemia, hypertension, and insulin resistance. Chronic inflammation with release of inflammatory mediators including tumor necrosis factor (TNF) and interleukins represent important pathogenic factor in the development of MS. The present work investigated the effect of TNF inhibitor; etanercept and IL-1β inhibitor; diacerein in treating high fructose-induced MS in rats. Rats were divided into 4 groups: normal control (received normal diet); high fructose fed (HF) (received 20% fructose plus saline to serve as control MS group; etanercept-treated group (received HF plus etanercept 0.3 mg/kg/day); and diacerein-treated group (received HF plus diacerein 50 mg/kg/day). Administration of drugs was started after 6 weeks and continued for 2 weeks to complete 8 weeks, the total period of the study. Visceral fat index (visceral fat weight /body weight ratio), insulin resistance , serum lipid profile (triglyceride (TG), high density lipoprotein (HDL)), malondialdehyde (MDA), reduced glutathione (GSH), catalase, uric acid, C-reactive protein (CRP), and tumor necrosis factor- α (TNF-α) were measured. The results showed that etanercept, but not diacerein, caused significant improvement in insulin resistance, and lipid profile. Such effect was associated with significant improvement in serum levels of oxidative stress markers (MDA, GSH, and catalase), and inflammatory markers (uric acid, CRP, and TNF-α). These results indicate that etanercept could be effective in treating high fructose-induced MS possibly via antioxidant and anti-inflammatory effects.

KEY WORDS:

Etanercept Diacerein Metabolic syndrome

INTRODUCTION:

Metabolic syndrome (MS) is a complex of medical disorders that, when occurring together, increased the risk of cardiovascular disease and T2D. It is a worldwide problem with high socioeconomic consequences (Lakka et al., 2002). The prevalence of the metabolic syndrome was 34.5 ± 0.9% (percent ± SE) among all participants, 33.7 ± 1.6% among men, and 35.4 ± 1.2% among women (Ford, 2005).

In a trial to find drug therapies for MS there is a big challenge necessi-tating the understanding of the cellular mechanisms underlying the metabolic abnormalities with MS. Insulin resistance and inflammatory status are proposed mechanisms of MS. Inflammatory status is derived largely from the secretory activity of adipose tissue, particularly intra-abdo-minal or visceral fat which increases with MS. Contrary to the former concept of fat as an inert tissue mass, adipocytes are increasingly being recognized as secretory entities, they release cytokines and other inflame-matory markers or signaling molecules

-- termed "adipokines"--include leptin, TNFα, ILs, resistin, and adiponectin. (Hotta et al., 2000).

Etanercept is a drug that acts as TNF inhibitor (Lee et al., 2003). It was reported to improve abnormal inflame-matory cardiovascular risk indices in patients at high risk for cardiovascular disease with the MS (Lakka et al., 2002).

Diacerein antagonizes IL-1β actions. Clinical studies of Ramos-Zavala et al., (2011) indicated increased insulin secretion and improved metabolic control (including IL-1β, TNFα, IL-6, and fasting insulin levels) after diacerein administration in patients with type 2 diabetes mellitus.

The present study investigated the effect of etanercept and diacerein in treatment of high fructose-induced MS in rats. The effect of tested drugs on the pathogenesis of MS had been examined.

MATERIALS AND METHODS:

Animals:

Adult male rats (150-200 g) obtained from the animal house (National Center of Research, El-Giza, Egypt). They were allowed free access to tap water and normal rats'diet (El-Nile Company, Egypt) for one week, as an adaptation. All experimental proto-cols were approved by the board of faculty of medicine, Minia Univer-sity and coincide with international guidelines.

Chemicals:

Fructose, etanercept, and diace-rein were obtained as powders from EL-Nasr Pharmaceutical Company; Wyeth Pharmaceuticals and Eva pharma companies, respectively.

Experimental design:

Animal grouping

Rats were divided into 4 groups, 6 rats each and treated as follows: group 1, a normal control which received normal diet; group 2, high fructose group (HF), this received 20% fructose (10% with diet (weight/weight) and 10% with water (weight/volume) and injected with saline to serve as control MS group (Faure et al., 1999).; group 3, etanercept-treated HF group (HF+Etan), received HF plus etanrecept (0.3 mg/kg, 3 times /week. s.c); and group 4, diacerein-treated HF group (HF+Dia), received HF plus dicerein (50 mg/kg/day, P.O). Administration of drugs was started after 6 weeks and continued for 2 weeks to complete 8 weeks, the total period of the study.

Sample collection:

At the end of the experimental period, the animals were weighted, anesthetized with ether. Blood samples were collected from the abdominal aorta, centrifuged at 5000 rpm for 10 minutes for serum separation, and kept at -80°C until further measurements.

Measurements:

Physical measurements

Visceral fat (adipose tissue surro-unding the abdominal and pelvic organs) was dissected and weighed. Visceral fat index was calculated according to the following equation: (visceral fat weight (g) /g body weight) x 100 (Hansen et al., 1997).

Biochemical measurements:

Fasting blood glucose and serum levels of fasting insulin, triglycerides (TG), high density lipoprotein (HDL), oxidative stress parameters (reduced glutathione (GSH), catalase, malon-dialadehyde (MDA) and inflammatory parameters (C-reactive protein (CRP),

tumor necrosis factor-alpha (TNF-α), and uric acid) were measured.

Insulin resistance:

Fasting blood glucose was measured using the ACCU-CHEK Active Blood Glucose Meter (Roche, Mannheim, Germany). Serum insulin was measured by enzyme-linked immunosorbent assay (ELISA) Kit (SPI-BIO, France) according to the Kit instruction. Insulin resistance (IR) was calculated using Homeostasis Model Assessment-insulin resistance (HOMA-IR) [fasting glucose (mg/dl) x fasting insulin (μIU/ml)] / 405 (Matthews et al., 1985).

Lipid profile:

Serum TG and HDL were determined using commercially available kits (Biodiagnostic, Giza, Egypt) and expressed as mg/dl according to the kit instructions and quantitated at 500 nm using Beckman-DU-64 spectrophotometer (USA).

Oxidative stress markers (Malondialdehyde (MDA), Reduced glutathione (GSH), and catalase)

Serum levels of MDA were measured according to the thiobarbituric acid method as previously described by Buege and Aust (1978). It depends on measuring MDA, the breakdown products of lipid peroxides. The absorbance was read at 535 nm and the corresponding concentration was calculated from a standard curve using 1,1,3,3-tetraethoxypropane as a standard.

Reduced glutathione (GSH) was measured using colorimetric kit (Biodiagnostic, Egypt) according to kit instructions. The method based on the reduction of 5,5' dithiobis (2-nitrobenzoic acid) (DTNB) with glutathione to produce a yellow compound. The reduced chromogen is directly proportional to GSH concentration and its absorbance was measured at 405 nm using Beckman-DU-64 spectrophotometer (USA).

Catalase activity in the serum was determined using colorimetric methods (Biodiagnostic, Egypt). Catalase reacts with a known quantity of H_2O_2. In the presence of peroxidase, the remaining H_2O_2 reacts to form a chromophore with color intensity inversely proportional to the amount of catalase in sample.

Determination of inflammatory markers (C-reactive protein, Tumor necrosis factor-α, and Uric acid)

Serum C-reactive protein (CRP) was measured by enzymatic colorimetric kits (Agappe Diagnostic LTD, India). Determination and semi-quantitation of CRP depends on rapid agglutination procedure.

Tumor necrosis factor-α (TNF- α) was measured by ELISA Kit (ID Labs Inc., Canada) according to manufacture instruction. It depends up on using wells coated with a polyclonal antibody specific for rat TNF-α. After incubation with the rat TNF-α antigen and a biotinylated polyclonal antibody and washing to remove the unbound enzyme, a substrate solution was added to induce a colored reaction product. The intensity of this colored product was directly proportional to the concentration of rat TNF- α present in the samples. The values were read at 450 nm in an ELISA reader.

Serum uric acid was determined using an enzymatic colorimetric kit (Biomed diagnostic, Egypt). The method was based on oxidation of uric

acid by uricase to allantoin and hydrogen peroxide. The hydrogen peroxide causes oxidative reaction in the presence of peroxidase, forming a red colored quinoneimine dye. The intensity of the color produced is directly proportional to the concentration o f uric acid in the sample, with maximum absorbance at 520 nm.

Statistical analysis of the data:

Results were expressed as means ± standard error of mean (SEM). One-way analysis of variance (ANOVA) followed by the Tukey-Kramar post analysis test was used to analyze the results for statistically significant difference. p values less than 0.05 were considered significant. Graph Pad Prism was used for statistical calculations (version 5.03 for Windows, Graphpad Software, San Diego California, USA, www.graphpad.com).

RESULTS:

Effect of etanercept and diacerein on body weight and visceral fat index

There was no significant difference in the body weight between groups. High fructose feeding increased significantly visceral index compared with normal control. Either Diacerein or etanercept significantly decreased visceral fat index to almost normal values (Table 1).

Effect of etanercept and diacerein on insulin resistance, and lipid profile

High-fructose intake significantly caused insulin resistance indicated by increased fasting glucose and insulin levels as well as by increased HOMA-IR compared to control value. Etanercept reduced significantly insulin resistance parameters to near control values (Table 1). Moreover, high-fructose intake produced dyslipidemia manifested by significant increase in serum TG and decrease serum HDL as compared to control group. Etanercept almost normalized the fructose-induced dyslipidemia (Table 1). Diacerein did not produce any significant change in all measured parameters (Table1).

Effect of etanercept and diacerein on serum levels of oxidative stress markers (MDA, GSH, and catalase)

In high fructose-fed rats, there was significant increase in MDA and decrease in GSH and catalase activity compared to normal control. Treatment with etanercept significantly attenuated the effect of high fructose intake on the measured oxidative stress parameters. Diacerein did not produce any significant change in oxidative stress markers compared with high fructose-fed group (Table 2).

Effect of etanercept and diacerein on serum level of inflammatory markers (CRP, uric acid, and TNF-α)

Serum CRP level significantly increased in high fructose-fed rats compared to normal control. Treatment with etanercept significantly decreased the effect of high fructose intake on CRP serum level (Table 2). diacerein did not produce any significant changes compared with high fructose-fed group (Table 2).

In high fructose-intake rats, there was significant increase serum uric acid compared to normal control. Treatment with etanercept significantly attenuated the effect of high fructose intake on serum uric acid (Table 2). Diacerein did not produce any significant changes compared with high fructose-fed group (Table 2).

In high fructose-fed rats, there was significant increase in serum TNF-α compared to control group. Etanercept almost restored the control values

(Table 2). Diacerein did not produce any significant change compared with high fructose-fed group (Table 2).

Table (1): Effect of etanercept and diacerein on metabolic disorders in fructose-induced metabolic syndrome

	Control	HF	HF + Etan.	HF + Dia.
BW (g)	222.30 ± 6.25	227.80 ±5.50	213.00 ±5.00	230.00 ±7.20
VW/BW ratio	0.12 ±0.01	4.38 ± 0.38[a]	0.81 ± 0.26[b]	0.89 ±0.18[b]
Fasting blood Glucose (mg/dl)	90.00 ± 3.82	134.00 ± 2.17 [a]	93.60 ± 3.08 [b]	121.00 ± 2.30[a]
Fasting serum insulin (μIU/ml)	12.2 ± 0.60	22.1 ± 1.70 [a]	12.6 ± 0.80 [b]	21.3 ± 1.40 [a]
HOMA-IR	3.10 ± 0.10	7.30 ± 0.70 [a]	3.30 ± 0.20 [b]	6.50 ± 0.40 [a]
Serum TG (mg/dl)	74. 7 ± 3.1	222.5 ± 8.8[a]	83. 7 ± 4.5 [b]	202.3 ± 8.6 [a]
Serum HDL (mg/dl)	53. 7 ± 2.7	27.3 ± 1.4 [a]	46.8 ± 4.4 [b]	27. 7 ± 1.7 [a]

Values represent the mean ± SEM. (n= 4-6). [a, b,] significantly different from control group, HF, etanercept and diacerein, respectively, at $p < 0.05$. HF = fructose fed; Etan.= etanercept; Dia. = diacerein. HOMA-IR = Homeostasis Model Assessment-insulin resistance [fasting glucose (mg/dl) x fasting insulin (μIU/ml)] / 405. BW = body weight. VW = visceral fat weight.
One-way analysis of variance (ANOVA) followed by the Tukey-Kramar post analysis test was used to analyze the results for statistically significant difference.

Table (2): Effect of etanercept and diacerein on serum levels of oxidative stress and inflammatory parameters in fructose-induced metabolic syndrome

	Control	HF	HF + Etan.	HF + Dia.
MDA (nmol/dl)	217.06 ± 13.14	384.90 ± 25.52[a]	238.30 ± 10.92[b]	363.28 ± 24.0 [a]
GSH (mg/dl)	54.56 ± 2.54	30.06 ± 1. 94 [a]	52.14 ± 2.85 [b]	30.29 ± 1.56 [a]
Catalase (U/dl)	34.7± 2.2	20.04 ± 1.6[a]	30.93 ± 3.5[b]	19.4 ± 1.7[a]
CRP (mg/dl)	1.56 ± 0.24	22.08 ± 1.9 [a]	5.6 ± 0.52 [b]	20.9 ± 1.3 [a]
Uric acid (mg/dl)	3.76 ± 0.24	7.56 ± 0.3 [a]	4.14 ± 0.197 [b]	6.58 ± 0.3 [a]
TNF-α (pg/ml)	12.09 ± 1.38	32.03 ± 1.8 [a]	13.27 ± 1.82 [b]	24.36 ± 2.16 [a]

Values represent the mean ± SEM. (n= 4-6). [a, b,] significantly different from control group, HF, etanercept and diacerein, respectively, at $p < 0.05$. HF = fructose fed; Etan.= etanercept; Dia. = diacerein.
One-way analysis of variance (ANOVA) followed by the Tukey-Kramar post analysis test was used to analyze the results for statistically significant difference.
GSH = Reduced glutathione, TNF- α = Tumor necrosis factor-α and CRP = C-reactive protein.

DISCUSSION:

High fructose feeding animal models are frequently used to understand the pathogenesis and therapeutic interventions of MS (Tran

et al., 2009). The results of the present study showed that high fructose feeding caused insulin resistance indicated by increased fasting glucose and insulin levels and HOMA-IR. It also produced dyslipidemia manifested by increased serum TG and decreased HDL. Additionally, it significantly increased the visceral fat index. These results are in consistence with previous studies (Faure et al., 1999).

Several explanations for the metabolic abnormalities in high fructose feeding have been put forward. Impaired activity of the carbohydrate metabolizing enzymes (Van Den Berghe, 1986) and increased oxidative stress (Fuare et al., 1999) have been reported. In the liver, fructose is metabolized into glyceroldehyde and dihydroxyacetone phosphate. These particular fructose end products converge with the glycolytic pathway. Of key importance is the ability of fructose to by-pass the main regulatory step of glycolysis, the conversion of glucose-6-phosphate to fructose 1,6-bisphosphate, controlled by phosphofructokinase. Thus, while glucose metabolism is negatively regulated by phosphofructokinase, fructose continuously enters the glycolytic pathway and promotes the overproduction of TG (Park et al., 1992). Excess TG with subsequent excess free fatty acids may cause insulin resistance by stimulating gluconeogenesis and activating protein kinase C (PKC) and Jun N-terminal kinase (JNK), which may interfere with tyrosine phosphorylation of insulin receptor substrates (IRS) (Dey et al., 2005).

In the present study, etanercept improved fructose-induced metabolic disorders. On the other hand, the present results showed that diacerein did not change the fructose-induced metabolic abnormalities.

In the present study, etanrecept decreased fasting blood glucose and fasting insulin, so it reduced insulin resistance, also it improved liver weight and decrease incidence of fatty liver. Stanley and his group (2011) reported that etanrecept demonstrated improvements in glucose level in obese patients. The improvements in fasting glucose and adiponectin after etanercept occurred in the absence of changes in body composition or lipid parameters, suggesting a direct effect of TNFα on these parameters. This finding had gained support from the results of Campanati and his coworkers (2012) in which etanercept, caused significant reduction fasting insulin levels, and HOMA index. On contrast, Dominguez et al., (2005) demonstrated that etanrecept failed to change insulin sensitivity. The absence of an effect on insulin sensitivity may be due to dosing duration, the choice of population or animals and the presence of more powerful determinants of insulin sensitivity. In the present study, etanrecept which is an anti-inflammatory agent significantly decreased the level of blood glucose and IR so decrease circulating FFA levels and level of triglyceride.

The foregoing results revealed that etanrecept treated group increased the level of GSH, catalase and decreased the level of MDA signifi-

cantly. Because etanrecept improves endothelial function and diminishes the activity and expression of NADPH oxidase, consequently it exerts a potent protective effect against lipid peroxidation and also reducing of MDA contributed to preservation of tissue glutathione levels (Tran**,** et al., 2009). Vaida Voevod et al., (2012) supported our results in the influence of the administration of a TNFα inhibitor on the oxidant/antioxidant balance in chronic venous insufficiency.

In our study, etanrecept reduced inflammatory mediators (CRP, uric acid and TNFα). The anti-inflammatory effects of etanercept are due to its ability to bind TNFα, preventing in this way the interaction of TNFα with cell surface receptors. Etanercept can modulate biological responses induced and mediated by TNFα, including the expression of adhesion molecules responsible for the migration of leukocytes, serum cytokine level and matrix metalloproteins (Di Paola et al., 2007). Our results were supported by the study of Bernstein et al., (2006) on effects of etanercept in patients with the metabolic syndrome. In the same way, Di Paola et al., (2007) demonstrated that treatment with etanercept attenuates, TNF α activity, infiltration of neutrophils, cell apoptosis, iNOS, and nitrotyrosine formation.

It seems puzzling that diacerein was not as effective which may be explained by the short duration of its administration (2 weeks after induction of MS for 6 weeks). This suggestion is supported by presence of tendency to improve the metabolic effects of fructose, however it didn't reach a significant level. Diacerein showed no significant decrease in IR, inflammation or oxidative stress. This means that diacerein needs prolonged period to be effective as a potential therapy in MS.

In conclusion, etanrecept might represent a potential therapy for MS. It effects was associated with reduction of IR, lipids profile, serum levels of TNFα, CRP and uric acid and improvement in oxidative stress.

REFERENCES:

1. Faure, P., Rossini, E., Wiernspeger, N., Richard, M.J., Favier, A., Halimi, S., 1999. An insulin sensitizer improves the free radical defense system potential and insulin sensitivity in high fructose-fed rats. Diabetes 48, 353-357.

2. Hansen, P.A., Han, D.H., Nolte, L.A., Chen, M., Holloszy, J.O., 1997. DHEA protects against visceral obesity and muscle insulin resistance in rats fed a high-fat diet. Am J Physiol 273, 1704-1708.

3. Matthews, D.R., Hosker, J.P., Rudenski, A.S., Naylor, B.A., Treacher, D.F., Turner, R.C., 1985. Homeostasis model assessment: insulin resistance and betacell function from fasting plasma glucose and insulin concentrations in man. Diabetologia 28, 412–419.

4. Ford ES (2005): Prevalence of the metabolic syndrome defined by the international diabetes federation among adults in the U.S., Diabetes Care, 28: 2745-2749.

5. Lakka HM, Laaksonen DE and Lakka TA (2002): The metabolic syndrome and total and cardiovascular disease mortality in middle-aged men. JAMA. 288: 2709-2716.

6. Lee H, Kimko HC, Rogge M, Wang D, Nestorov I and Peck CC (2003): Population pharmacokinetic and pharmacodynamic modeling of etanercept using logistic regression

analysis. Clin Pharmacol Ther. 73:348-365.

7. Hotta K, Funahashi T, Arita Y, Takahashi M, Matsuda M, OkamotoY, Iwahashi H, Kuriyama H, Ouchi N and Maeda K (2000): Plasma concentrations of a novel, adipose-specific protein, adiponectin, in type 2 diabetic patients. Arterioscler Thromb Vasc Biol. 20: 1595–1599.

8. Ramos-Zavala MG, González-Ortiz M, Martínez-Abundis E and Robles-Cervantes JA, González-López R and Santiago-Hernández NJ (2011): Effect of diacerein on insulin secretion and metabolic control in drug-naive patients with type 2 diabetes: a randomized clinical trial. Diabetes Care, 34:1591-1594.

9. Tran LT, MacLeod KM and McNeill JH (2009): Chronic etanercept treatment prevents the development of hypertension in fructose-fed rats. Mol Cell Biochem. 330: 219-228.

10. Van den Berghe G (1986): Fructose: metabolism and short-term effects on carbohydrate and purine metabolic pathways. Prog Biochem Pharmacol. 21: 1–32.

11. Park O, Cesar D, Faix D, Wu K, Shackleton C, Hellerstein M (1992): Mechanism of fructose-induced hypertriglyceridemia in the rat. Activation of hepatic pyruvate dehydrogenase through inhibition of pyruvate dehydrogenase kinase. Biochem J 282: 753–757.

12. Stanley TL, Zanni MV, Johnsen S, Rasheed S, Makimura H, Lee H, Khor VK, Ahima RS, and Grinspoon SK. (2011): TNF-antagonism with etanercept decreases glucose and increases the proportion of high molecular weight adiponectin in obese subjects with features of the metabolic syndrome. J Clin Endocrinol Metab. 96: 146-150.

13. Campanati A, Ganzetti G, Sario A Di, Damiani A, Sandroni L, Rosa L, Benedetti A and Offidani A (2012): The effect of etanercept on hepatic fibrosis risk in patients with non-alcoholic fatty liver disease, metabolic syndrome, and psoriasis. J Gastroenterol.249:239-252.

14. Dominguez H, Storgaard H, Rask-Madsen C, Steffen H T, Ihlemann N, Baunbjerg ND, Spohr C, Kober L, Vaag A and Torp-Pedersen C. (2005): Metabolic and vascular effects of tumor necrosis factor-alpha blockade with etanercept in obese patients with type 2 diabetes. J Vasc Res. 42:517–525.

15. Vaida Voevod CM, Moldovan R, Decea N and Muresan A (2012): Influence of the administration of a TNF-αInhibitor on the oxidant/ antioxidant balance in chronic venous insufficiency. Applied Medical Informatics, 30: 32-38.

16. Di Paola R, Mazzon E, Muià C, Crisafulli C, Terrana D, Greco S, Britti D, Santori D, Oteri G, Cordasco G and Cuzzocrea S (2007): Effects of etanercept, a tumour necrosis factor-α antagonist, in an experimental model of periodontitis in rats. Br J Pharmacol. 150:286-297.

17. Bernstein EL, Berry J, Kim S, Canavan B and Steven SK (2006): Effects of etanercept in patients with the metabolic syndrome. Intern Med. 166: 902–908.