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Effect of infliximab and tocilizumab on fructose-induced hyperinsulinemia and hypertension in rats

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ABSTRACT

Fructose administration can induce hypertension, insulin resistance and hypertriglyceridemia. Here, we investigated the possible protective effect of infliximab (IFX), a tumor necrosis factor alpha (TNF-α) inhibitor, or tocilizumab (TOC), an interleukin-6 (IL6) inhibitor, on fructose-induced increase in blood pressure, insulin resistance and hyperlipidemia in rats. The animals were fed a 60% fructose diet in the absence or presence of IFX (5 mg/kg, i.p., once weekly) or TOC (8 mg/kg, i.p., once every two weeks). Fructose significantly increased blood pressure, heart rate and homeostatic model assessment of insulin resistance (HOMA-IR). Fructose also significantly raised the concentrations of fasting plasma insulin, triglycerides, total cholesterol, uric acid, tumor necrosis factor–alpha (TNF-α), interleukin 6 (IL-6), malondialdhyde (MDA) and nitric oxide. Fructose also significantly decreased plasma superoxide dismutase (SOD) and catalase activities. In addition, fructose significantly increased aortic endothelin and nitric oxide concentrations. Both IFX and TOC attenuated the fructose-induced increase in blood pressure, insulin resistance, and the concentrations of uric acid, MDA and IL-6. TOC significantly reduced fructose-induced increase in triglycerides and cholesterol. In addition, IFX increased plasma SOD and catalase activities. Our results showed that both IFX and TOC were partially successful in reversing fructose – induced changes.

1. Introduction

Metabolic syndrome (MS) represents a clustering of cardiovascular risk factors that include abdominal obesity, dyslipidemia, insulin resistance and hypertension [1]. Fructose is a monosaccharide found naturally in only a few foods, primarily fruits, and is typically consumed as sucrose or as a component of high-fructose corn syrup [HFCS] [2]. The consumption of fructose, from either beverages or food, has increased globally [3]. Clinical trials provided evidence that consumption of sucrose or HFCS increases several risk factors for cardiovascular disease and metabolic syndrome [4]. Inflammation is now recognized as a key factor of cardiometabolic diseases associated to obesity and insulin resistance, especially type 2- diabetes, metabolic syndrome, and cardiovascular diseases [5]. Chronic inflammation and endothelial dysfunction are commonly observed in the metabolic syndrome [6]. Targeting inflammation may represent a valuable add-on therapy in the management of metabolic syndrome [5]. There are many animal models for inducing metabolic syndrome experimentally [7]. Fructose administration, either in the drinking water (10%), or in the diet (60–66%) has been reported to induce systemic hypertension, hypertriglyceridemia and endothelial dysfunction in rats [1,8–10]. Esser et al [5] recently reviewed the data regarding the role of the inflammatory cytokines; tumor necrosis factor-alpha (TNF-α), interleukin-6 (IL-6), interleukin 1β (IL-1β), nuclear factor-kappaB (NFkB) in the pathophysiology of inflammation and metabolic syndrome, and have concluded that further evidence is required to establish the role of anti-inflammatory drugs in the management of cardiometabolic disorders.

The aim of this study was to examine the effect of infliximab, a known TNF-α inhibitor [11], and tocilizumab, a recombinant humanized anti-IL-6R monoclonal antibody that blocks IL-6-mediated signaling pathway by binding to both IL-6R [12] on fructose – induced hypertension and insulin resistance in rats.

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Table 1
Effect of infliximab (IFX) and tocilizumab (TOC) on physiological parameters in fructose (60%) fed male Wister rats.

Variables	Control	Fructose	Fructose + IFX	Fructose + TOC
Initial body weight (g)	242 ± 14	243 ± 16	243 ± 12	243 ± 15
Final body weight (g)	348 ± 11	295 ± 12	307 ± 8	305 ± 21
Body weight change (%)	46.16 ± 10.01	22.24 ± 3.69	27.48 ± 4.81	25.67 ± 3.62
Water intake (mL)	19.17 ± 1.54	8.33 ± 1.23[a]	8.33 ± 1.23[a]	6.67 ± 1.05[a]
Urine output (mL))	11.17 ± 0.91	5.67 ± 0.76[a]	6.00 ± 0.77[a]	5.33 ± 0.67[a]

$P < 0.05$ is considered significant.
[a] Significant difference from control group.

2. Materials and methods

2.1. Animals

Wister male rats (180–290 g) were housed in a room at a temperature of 22 ± 2 °C, relative humidity of about 60%, with a 12 h light–dark cycle (lights on at 6:00), and fed *ad libitum* standard or 60% fructose diet and tap water. The protocols were approved by the Medical Research Committee, College of Medicine and Health Sciences (IG/MED/PHAR/16/02) in accordance with guidelines of Sultan Qaboos University Animal Research Ethics committee. All procedures involving animals and their care were carried out in accordance with the guidelines of international laws and policies (EEC Council directives 2010/63/EU, 22 September 2010 and NIH Guide for the Care and Use of Laboratory Animals, NIH Publications, 8th edition, 2011).

2.2. Experimental design

Wister male rats (n = 24) were randomly distributed into four equal groups and treated as follows:

Group 1: Control: fed standard diet for 8 weeks and given intraperitoneal injection of saline every week starting from the 5th week until the end of the 8th week.

Group 2: Fructose: fed 60% fructose diet for 8 weeks and given intraperitoneal injection of saline every week starting from the 5th week until the end of the 8th week.

Group 3: Infliximab: fed 60% fructose diet for 8 weeks and given intraperitoneal injection of infliximab (5 mg/kg) once every week starting from the 5th week until the end of the 8th week.

Group 4: Tocilizumab: fed 60% fructose diet for 8 weeks and given intraperitoneal injection of tocilizumab (8 mg/kg) once every two weeks starting from the 5th week until the end of the 8th week.

Blood pressure and heart rate of conscious rats were measured at the beginning of the experiment and at the end of week 8 by the tail-cuff method [Blood Pressure Analysis System™ (BP-2000 SERIES *II*, Visitech Systems, Apex, NC, USA)]. Before sacrifice, rats were placed individually in metabolic cages (Tecniplast, Italy) to collect the urine voided in the last 24 h. At the end of the experiment and after 16 h fast, the rats were anesthetized with ketamine (75 mg/kg) and xylazine (5 mg/kg) intraperitoneally. Blood (about 4–5 mL) was collected from the abdominal aorta and centrifuged at 900*g* at 4 °C for 15 min to separate plasma. The plasma was stored at −80 °C pending analysis.

Aorta were excised, blotted on filter paper and weighed. Parts of aorta were quickly dipped in liquid nitrogen and then kept frozen at −80 °C for conducting biochemical analysis. Homogenization of aorta was done in cold PBS buffer, 1:30 ratio (5 mg/150 µl) by ULTRA-TURRAX homogenizer in ice. After this, homogenate was centrifuged for 10 min in a micro centrifuge, at 5000 rpm and temperature at 4 °C. Supernatant was collected for analysis.

The doses of infliximab and tocilizumab were chosen according to previous studies [13,14], and also our own preliminary experiments.

2.3. Biochemical measurements

Plasma triglycerides, total cholesterol, HDL-C, LDL-C, uric acid were measured using fully automated chemistry analyzer BS-120, MINDRAY (Shenzhen, China). Fasting plasma insulin was measured by the Thermo Scientific Pierce™ Rat Insulin ELISA™ kit. Blood glucose was measured by ONETOUCH Select Test strip using a glucometer (Free Style Optium, Abbott Diabetes Care Ltd, Oxen, UK). Homeostatic model assessment of insulin resistance (HOMA-IR) was calculated as fasting blood glucose (mmol/L) X fasting blood insulin (IU/L) /22.5. HOMA-IR is a valid measure to determine insulin resistance in Wister rats [15]. Measurement of aortic endothelin-1 was done by ELISA kit (Quantikine ELISA Endoelin-1 Immunoassay R & D systems) and plasma and aortic nitric oxide by total Nitric oxide (Parameter, R & D systems). Plasma tumor necrosis factor alpha (TNF-α) and interleukin-6 were measured using ELISA kits from Abcam (Cambridge, UK). Plasma superoxide dismutase activity (SOD) was measured using a colorimetric method (BioVison, CA, USA). Plasma catalase was measured using a colorimetric catalase assay kit from Abcam (Cambridge, UK). Plasma MDA was measured using a colorimetric assay kit (Biovision, CA, USA).

2.4. Statistical analysis

Data were expressed as means ± SEM, and were analyzed with GraphPad Prism Version 5 for Windows software (Graphpad Software Inc., San Diego, USA). Comparisons between the four groups were performed by one way analysis of variance ANOVA, followed by Bonferroni comparisons. *P* values < 0.05 were considered significant.

3. Results

3.1. Effect of infliximab and tocilizumab on physiological parameters in fructose-treated rats

Table 1 shows that fructose did not significantly affect the increase in body weight when compared to the control group. Fructose treatment reduced both water intake and urine output. Neither IFX nor TOC had any significant effect on the changes induced by fructose.

3.2. Effect of infliximab and tocilizumab on blood pressure and heart rate in fructose-treated rats

There were no significant differences in baseline systolic blood pressure (130 ± 4; 132 ± 4; 133 ± 4; 137 ± 3 mmHg), mean arterial blood pressure (86 ± 3; 89 ± 23; 89 ± 2; 90 ± 4; 89 ± 2 mmHg) and heart rate (393 ± 7; 389 ± 6; 396 ± 9; 387 ± 9 beats/min) between the four groups. Fig. 1 shows that fructose caused an increase in systolic blood pressure, mean arterial blood pressure and heart rate at the end of week 8 when compared to control rats. IFX reduced systolic blood pressure, mean arterial pressure and heart rate. Tocilizumab significantly reduced mean arterial pressure and heart rate but the reduction in systolic blood pressure was not significant.