International Immunopharmacology xxx (2015) xxx–xxx

Contents lists available at ScienceDirect

International Immunopharmacology

journal homepage: www.elsevier.com/locate/intimp

Diethylcarbamazine citrate ameliorates insulin resistance in high-fat diet-induced obese mice via modulation of adipose tissue inflammation

Mahmoud Abdel-Latif

Zoology Department, Faculty of Science, Beni-Suef University, 62511 Salah Salem Street, Beni-Suef, Egypt

ARTICLE INFO

Article history:
Received 20 April 2015
Received in revised form 17 September 2015
Accepted 23 September 2015
Available online xxxx

Keywords:
Diethylcarbamazine citrate
Insulin
Adipose tissue
Inflammation

ABSTRACT

Diethylcarbamazine citrate (DEC) had been known as anti-inflammatory drug but its effect on obesity-induced insulin resistance as a result of released inflammatory mediators from adipose tissue (AT) was not known. White male albino mice were fed with high fat diet (HFD) for 18 weeks to induce obesity. DEC at different three doses (12, 50 and 200 mg/kg) was orally administered twice a week starting at week 6. Body, liver and adipose tissue weights were taken, while glucose tolerance, insulin resistance, blood triglycerides and levels of adipokines (leptin, TNF-α, IL-6 and MCP-1) were tested. The activity of cyclooxygenase (COX) in the liver tissue homogenate was also tested. In addition, NF-κBp65 localization in liver cell cytoplasmic and nuclear fractions was detected using Western blotting. The only effective anti-inflammatory dose was 50 mg/kg to reduce ($p < 0.05$) the high levels of glucose, insulin and triglycerides in serum. DEC was not anti-obesity drug because the weights of body, liver and adipose tissues were not changed. Hyperleptinemia was decreased ($p < 0.001$) and associated with a reduction in serum levels of TNF-α, IL-6 and MCP-1 ($p < 0.001$). In addition, the activity of COX in DEC treatment decreased significantly ($p < 0.01$), while NF-κBp65 localization in nuclear extracts was obviously inhibited in 50 mg/kg treated group. It could be concluded that DEC was the only effective dose against mouse insulin resistance but not lipid accumulation.

1. Introduction

Obesity is a growing public health problem and its prevalence has reached epidemic proportions in recent decades [1]. Obesity and associated metabolic syndrome are accompanied by heightened levels of pro-inflammatory mediators, not only systemically but also locally in metabolically critical tissues such as AT, liver and skeletal muscle [2]. A plethora of studies has shown that an increase in proinflammatory mediators such as tumor necrosis factor (TNF)-α, interleukin (IL)-6 and macrophage chemotactic protein (MCP)-1 and a decrease in released adiponectin were evident [3–5]. These dysregulations of adipokine secretory patterns from AT result in adipocyte lipolysis and hepatic steatosis, eventually leading to systemic leptin- and insulin resistance [6]. Major changes of immune cell populations – especially of macrophages – within adipose tissue play a key role in propagating obesity-induced adipose tissue inflammation [7,8].

Diethylcarbamazine (DEC) interferes with arachidonic acid (AA) metabolism for the clearance of microfilariae in *Wuchereria bancrofti* infected individuals. It inhibits prostaglandins for the clearance of blood microfilariae [9]. DEC has been known as an anti-inflammatory drug because of its success to reduce the experimentally induced inflammation in lung and liver [10,11]. These reduced inflammatory responses could be attributed to the inactivation of NF-κB and thus suppressing the induction of NF-κB-dependent genes. DEC has also been known as an inhibitor for both COX and lipoxygenase (LO) pathways [12]. The drug was found to reduce hepatic inflammation and injury through reduction of inflammatory mediators and elevation of IL-10 [13].

Because of the anti-inflammatory effects of DEC, it was hypothesized that the drug can prevent obesity-linked adipose tissue inflammation and associated insulin resistance. This study aimed to test the effect of DEC at different doses on the levels of released adipokines and insulin resistance. The results could indicate that 50 mg/kg dose was the most effective.

2. Materials and methods

2.1. Animals and experimental protocol

Five-week-old male outbred Swiss mice (n = 60) at 10 g body weight were obtained from the National Institute of Ophthalmology, Giza, Egypt, housed five in a cage in a standard experimental animal laboratory and maintained under specific pathogen free conditions [14]. Animals were kept under controlled conditions of light (12 h light–dark cycle with lights on at 6 am) and temperature (24 ± 1 °C) without any stressful causes. All mice received water and food ad libitum. The mice were offered normal pellet diet (NPD) for a 1-week adaptation period and were then divided randomly into five groups consisting of 12

E-mail address: mahmoud_1232000@yahoo.com.

http://dx.doi.org/10.1016/j.intimp.2015.09.021
1567-5769/

mice each. One group was offered NPD while the other 4 groups were offered high fat diet (HFD) containing 58% fat, 25% protein and 17% carbohydrate, as a percentage of total kcal [15]. Feeding on NPD or HFD continued for 18 weeks [16] where the body weight was monitored at two weeks intervals. After six weeks of dietary manipulation, the 4 HFD-fed groups were treated at gavage with 0, 12, 50 and 200 mg of DEC in normal saline/kg body weight twice a week for a period of 12 weeks. At the end of 18 weeks period, the animals were euthanized after blood collection by decapitation. Blood was collected from the orbital sinus into tubes, kept on ice for 10 min, centrifuged at 3000 ×*g* for 20 min, and serum samples were collected and stored at −80 °C for subsequent analyses. Epididymal (gonadal), perirenal fat pads and liver were dissected out, weighed and directly stored at −80 °C for subsequent analyses. Liver tissue was homogenized in 0.1 M Tris–HCL, pH 7.8 containing 1 mM EDTA, centrifuged 3000 ×*g* for 20 min at 4 °C and supernatants were taken and stored at −80 °C. This study was approved by the Research Ethics Committee of Beni-Suef University. Experimental procedures are in accordance with Principles of Laboratory Animal Care formulated by the National Institutes of Health (National Institutes of Health Publication number 96-23, revised 1996).

2.2. Oral glucose tolerance test (OGTT)

This was performed at the end of 18 weeks in the same set of mice. Following a 6-h period of feed deprivation, 5 μL of tail blood was used to measure the blood glucose levels using the One Touch Ultra glucometer. Next, 2 g/kg body weight of 20% D-glucose was injected orally. Serial blood glucose measures were taken at 30, 60, and 120 min after the injection.

2.3. Serum insulin, adipokines and triglycerides

Commercially available ELISA kits were used to measure serum insulin (Raybiotech, Norcross, USA), leptin, TNF-α, IL-6 and MCP-1 (R & D systems, Minneapolis, USA). Homeostasis model assessment for insulin resistance (HOMA-IR) values was estimated as follows: HOMA-IR = [fasting insulin (μU/mL) × fasting glucose (mmol/L)]/22.5 [17]. The serum triglycerides were measured by an enzymatic colorimetric method using commercial kit (Spinreact, Girona, Spain). All assays were performed according to the manufacturers' protocols.

2.4. Cyclooxygenase activity assay

The method had been performed in a clear plastic 96-well plate [18]. Briefly, stock solutions from N,N,N′,N′-tetramethyl-p-phenylenediamine (TMPD; Sigma) in dimethyl sulfoxide (DMSO) at 20 mM, porcine hematin (Sigma) in 1 M NaOH (40 mM) and AA (Sigma) in 98% ethanol (1 mM). The reaction mixture contained final concentrations of TMPD at 100 μM, hematin at 1 μM and AA at 100 μM in Tris/HCl buffer (100 mM, pH 8). To 0.2 ml of reaction mixture/well, 10 μL of liver tissue homogenate was added and absorbance was read at 590 nm after 5 min. The absorbance in the absence of AA was subtracted from the absorbance in the presence of AA and the net enzyme activity (expressed as the change in OD at 590 nm, ΔA590) is then calculated. One unit is defined as the amount of enzyme that will catalyze the oxidation of 1.0 nmol of TMPD per minute at 25 °C. COX activity in units was calculated as follows:

COX activity = $\Delta A590 \div 10\ \text{min} \div 0.00826\backslash\text{mu}\ M^{-1} \div 0.01^{\#}\ \text{mL} \times 1000 \div 2^{\$}$ = nmol/min/mL (U/mL).

ΔA590 is the difference in absorbance between wells containing complete mixture and control wells without AA.

$^{\$}$Two molecules of TMPD are required for the reduction of PGG2 to PGH2.

$^{\#}$0.01 mL is the enzyme sample volume.

2.5. Preparation of cytoplasmic and nuclear extracts

Cytoplasmic and Nuclear extracts were prepared according to the previously described method [19]. Livers were homogenized in a Potter-Elvehjem homogenizer (Braun, Melsungen, Germany) with a loose-fitting Teflon pestle at 1000 rpm with eight up and-down strokes in ice-cold homogenization medium (250 mM sucrose, 5 mM $MgCl_2$, 10 mM Tris–HCl, pH 7.4), called buffer A. After filtration, the homogenate was centrifuged at 600 ×*g* for 10 min at 4 °C in a Beckman TJ-6 centrifuge (Beckman Instruments; Munich, Germany). To prepare the cytoplasmic extract the obtained supernatant was centrifuged at 100.000 ×*g* at 4 °C for 30 min in a Beckman L5-65B ultracentrifuge (swinging-bucket rotor). The pellet obtained by the first centrifugation was resuspended in buffer A and again centrifuged at 600 ×*g* at 4 °C for 10 min. This pellet of crude nuclei obtained after the second centrifugation was resuspended in 9 volumes of buffer B (2.2 M sucrose, 1 mM $MgCl_2$, 10 mM Tris–HCl, pH 7.4), homogenized, and centrifuged at 70.000 ×*g* at 4 °C for 80 min in a Beckman L5-65B ultracentrifuge. The purified nuclei were resuspended in buffer A.

2.6. Western blotting analysis

The protein quantity of cytoplasmic and nuclear extracts was performed using Bicinchoninic acid kit (Sigma). Twenty micrograms protein for detection of NF-κBbp65 was separated in SDS-10% polyacrylamide gels and transferred to nitrocellulose membranes (0.45 μm; Heidelberg; Serva Electrophoresis GmbH, Germany) by electroblotting. Membranes were washed in PBS/Tween buffer (PBS containing 0.3% Tween-20) and incubated for 1 h at room temperature (RT) in blocking buffer containing 5% non-fat milk in PBS/Tween-20, followed by washing and incubation with the anti-mouse NF-κBp65 (1:1000; AbD Serotec, Puchheim, Germany) in the same buffer overnight at RT. The immunocomplexes were detected by using horseradish peroxidase-labeled rabbit anti-mouse antibody (1:5000; KPL, Maryland, USA). After 2 h of incubation at room RT, bands were developed by adding substrate (50 mg 3,3′-Diaminobenzidine tetrahydrochloride and 100 μL H_2O_2 in 100 ml PBS). The intensity of immunoreactive bands was quantified by densitometry using NIH-Image software (version 1.59; National Institutes of Health, Bethesda, MD).

2.7. Statistical analysis

SPSS (version 20) statistical program (SPSS Inc., Chicago, IL) was used to carry out a one-way analysis of variance (ANOVA) on our data. When significant differences by ANOVA were detected, analysis of differences between the means of the treated and control groups were performed by using Dunnett's t test.

3. Results

3.1. Body, adipose tissues and liver weights

The body weight in HFD was significantly higher ($p < 0.001$) than NPD while the treated group with 50 mg DEC/Kg did not show any significant changes in comparison to HFD (Fig. 1). Similarly, adipose tissues (epididymal and perirenal) and liver weights in HFD were significantly ($p < 0.01$ and $p < 0.05$, respectively) higher than NPD but no significant reduction appeared in treated group (Fig. 2).

3.2. Effect of DEC on serum glucose, triglycerides and insulin

OGTT showed that the hyperlipidic diet promoted an increase at all time points when compared to control (HFD versus NFD). The AUC (area under the curve) analysis increased significantly ($p < 0.01$) in HFD compared with NFD (Fig. 3). The dose 50 mg/kg of DEC was only effective to reduce the glucose levels at all the time points. The AUC

Please cite this article as: M. Abdel-Latif, Diethylcarbamazine citrate ameliorates insulin resistance in high-fat diet-induced obese mice via modulation of adipose tissue inf..., Int Immunopharmacol (2015), http://dx.doi.org/10.1016/j.intimp.2015.09.021