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Evaluation of metabolic and immunological changes in streptozotocin-nicotinamide induced diabetic rats

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ABSTRACT

Type 2 diabetes is a chronic disease with growing public health concern globally. Finding remedies to assist this health issue requires recruiting appropriate animal model for experimental studies. This study was designated to evaluate metabolic and immunologic changes in streptozotocin-nicotinamide induced diabetic rats as a model of type 2 diabetes. Male rats were induced diabetes using nicotinamide (110 mg/kg) and streptozotocin (65 mg/kg). Following 42 days, biochemical and immunological tests showed that diabetic rats had higher levels of blood glucose, WBC, certain abnormalities in lipid profile and insufficient mitogenic responses of lymphocytes ($p < 0.05$). However, the status of the total antioxidant, inflammatory biomarkers and other parameters of full blood count (except HCT) were not significantly altered. Phenotyping assay indicated insignificant lymphocyte subtype imbalances excluding a significant rise in the level of CD4+CD25+ marker ($p < 0.05$). This model of diabetic animals may represent some but not all symptoms of human type 2 diabetes.

1. Introduction

Diabetes mellitus is the most common metabolic disorder affecting humans and is characterized by chronic hyperglycemia. The prevalence of diabetes worldwide was recently estimated to be 285 million people, corresponding to 6.4% of the world's adult population, which is expected to reach 552 million in 2030 [1]. Autoimmune insulitis leading to an absolute absence of insulin is known as type 1 while a combination of insulin resistance and a β-cell secretory deficiency is known as type 2 diabetes. The rate of type 2, which accounts for nearly 90% of all cases, is increasing [2].

Animal models are used in experiments for a better understanding of pathogenesis, finding new remedies and rehabilitations. Choosing an appropriate method for diabetes induction is crucial for more reliable outcomes. Several methods have been used to induce diabetes mellitus in rodent models that may have some benefits and limitations. Aloxan (2,4,5,6-tetraoxypyrimidine; 5,6-dioxyura-cil) and streptozotocin (STZ: 2-deoxy-2-(3-(methyl-3-nitrosoureido)-D-glucopyranose) are toxic substances to the pancreas with diabetogenic properties. Streptozotocin is used to induce both insulin-dependent and non-insulin-dependent diabetes mellitus (IDDM and NIDDM, respectively) [3]. Meanwhile, diet induction of type 2 diabetes such as long-term high-fat, high-sucrose diet [4], high-fat diet combined with STZ injection [5] and high fructose diet [6] are also appropriate in animal experiments.

Administration of both STZ and nicotinamide (NA pyridine-3-carboxamide) to induce diabetes is another model first presented in 1998 [7], STZ was shown to exert cytotoxic action on pancreatic β cells, while NA had partially protected β cells against STZ [8]. The current study was aimed to evaluate biochemical changes in NA-STZ induced diabetic rats, and to further elucidate alteration of immune system.

2. Materials and methods

2.1. Animals and diabetic induction

Male albino white rats from Sprague–Dawley (SD) species were acclimatized to laboratory conditions, *ad libitum* access to food and tap water (25 ± 3 °C temperature, 50–55 ± 5% humidity, and a 12 h light–dark cycle), for at least 7 days prior to commencement of the

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experiments. They were maintained on a regular commercial diet (Gold Coin, Malaysia) (14% fat, 61% carbohydrate, and 25% protein from total energy). All the experimental procedures were approved by the Animal Care and Use Committee (Reference No. UPM/FPSK/PADS/BR-UUH/00442) of the Faculty of Medicine and Health Sciences, Universiti Putra Malaysia.

Two experimental groups ($n = 16$) with 2 months old and 224 ± 5.3 g of initial body weight were compared as diabetic and healthy rats. The diabetic group was injected a single intraperitoneal dose of 110 mg/kg body weight nicotinamide in physiological saline, and 15 min later, they were injected 65 mg/kg body weight stereptozotocin dissolved in citrate buffer (pH 4.5) intraperitoneally [9]. Six weeks after the induction of diabetes, rats were examined for blood and immunological parameters. Body weight and food intake of rats were measured weekly.

2.2. Biochemistry tests

The blood taken from the tail vein of the diabetic rats was measured for fasting capillary blood glucose concentrations on days 0, 14, 21, 28 and 42 (Accu-chek Glucometer, *advantage II*). Following this, fasting plasma glucose, lipid profile (total cholesterol, LDL-c, HDL-c and triglyceride) and c-reactive protein (CRP) were also measured (automatic chemical analyser, Hitachi, Japan). Total antioxidant status (TAS) was assayed with Selectra E chemical analyser (Germany). Blood sample in EDTA tubes was used to check the haematology parameters, including RBC, WBC, HCT, HGB), MCV, MCH, MCHC, PLT (Sysmex hematology analyzer KX-21- Sysmex).

2.3. Immunological tests

Immunologic tests were performed by measuring serum interlukein-6 and tumor necrosis factor-α on days 0 and 42 (Sandwich ELISA, eBioscience, Austria). On day 42, spleens were collected from the sacrificed rats and further processed to isolate the splenocytes using standard procedures [10]. A hundred thousand splenocytes were plated in each well of 96-well plates. Splenocytes were stimulated with PHA and LPS respectively at two concentrations (1 and 5 μg/ml) and left for incubation for 72 h. Cultures were pulsed with 0.5 μCi of 3^H-thymidine at 24 h of incubation and later harvested onto glass filter mats by 96-well plate automated cell harvester (Harvester Mach III M, TOMTEC). The filter mat was dried in an oven (120 °C) for 10 min before adding 5 ml scintillation fluid (OptiPhase SuperMix Cocktail; Perkin Elmer, Boston USA). The filter mat was then sealed and fit into a scintillation cassette for radioactive measurement using a luminescent Microbeta counter (Wallac) [11]. Results were expressed as counts per minute (CPM).

2.4. Flowcytometery analysis

Flow cytometery analysis was performed to measure the expression of cell surface markers on splenocytes. A total of 1×10^6 cells from each rat were assessed for the expression of CD25-FITC, CD4-PE, CD3-APC, CD45RA-FITC, and CD161a-PE. The percentage of total T, B and NK cells was measured by a commercially available TBNK cocktail. All antibodies and cocktail kits were purchased from Becton Dickinson, USA. LSRFortessa II flow cytometer was used to acquire the samples and data were analyzed using FACS Diva software. The relevant isotype antibody controls were used in parallel to all measurements to set negative gating [12].

2.5. Statistical analysis

All data were analyzed by calculating their means and standard errors of means (SEM). Independent samples *t*-test was applied and datFlow cytometery analysis was performed to a were considered statistically significant when $p < 0.05$. All the statistical analyses were performed using SPSS version 20.

3. Results

Male healthy SD rats showed no significant difference in weight gain as compared with the diabetic rats (Fig. 1). The rising trend in body weight among the diabetic rats was rather slower than the normal rats. The capillary blood glucose in diabetic rats was found to be significantly high throughout the study. The baseline for fasting blood glucose for diabetics was 16.7 ± 2.5 (mmol/L) vs. 4.8 ± 0.3 (mmol/L) for non-diabetics. In diabetic rats, blood glucose gradually increased till day 28 of experiment; followed by a slight decline in the final 2 weeks. The mean differences between the two groups remained significantly different ($p < 0.05$) (Fig. 2).

Table 1 illustrates levels of blood biochemical tests in the NA-STZ diabetic and normal rats following 6 weeks of diabetic induction. Among the measured parameters, capillary blood glucose and plasma blood glucose were observed to be higher in diabetic rats compared with those in normal rats (53.2%). Also unlike normal rats, significant increase ($p < 0.05$) was found in triglyceride and LDL-c levels of diabetic rats. Total cholesterol, atherogenic indexes (1 and 2), HDL-c and TNF-α levels were insignificantly ($p > 0.05$) higher in diabetic-induced rats. However, IL-6 and CRP showed no alteration following administration of NA and STZ ($p < 0.05$). TAS decreased in the diabetic rats ($p > 0.05$). Changes in full blood count markers are demonstrated in Table 2. Although there were no significant differences ($p > 0.05$) between the two groups in RBC, HGB, MCV, MCH, MCHC and PLT, the levels in diabetic rats were lower than in normal rats. The only significant differences were observed for levels of WBC and HCT ($p < 0.05$).

3.1. Immunological changes

In order to determine whether diabetes induction alters lymphocyte proliferation, tritium thymidine incorporated cell proliferation assay was carried out in response to two concentrations of PHA and LPS stimulation (Fig. 3). Following proliferation with lowest concentration of mitogen, lymphocytes in both unstimulated ($p = 0.001$) and stimulated states ($p = 0.031$ for LPS and $p = 0.001$ for PHA) significantly failed to respond. Once the levels of stimuli increased to 5 μg/ml, cells showed more proliferation, yet a noticeable decline was recorded in diabetic rats ($p > 0.05$).

The flowcytometer analysis of total T, B and NK cells percentage indicated that no significant changes were observed between diabetic induced and normal rats (Table 3), following diabetes induction. The effects of diabetes induction on the percentage of regulatory T cells and T helper cells population were analyzed. Regulatory T cells which were determined by the level of expression CD25 increased in the diabetic rats compared with the normal rats

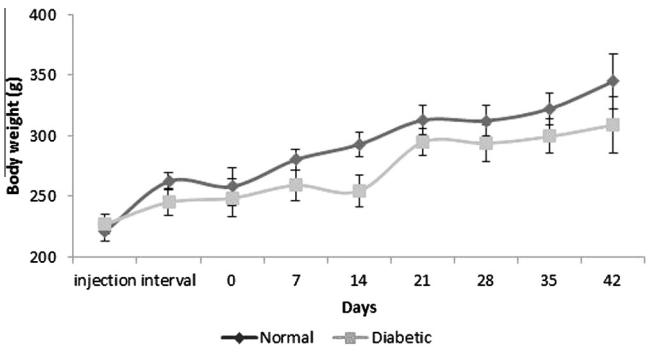

Fig. 1. Body weight measurement of healthy and diabetic rats.