Abstract

The existence of experimental animal model helps in the understanding of pathophysiology of diabetes and facilitates in the development of drugs for its treatment. Chemical induction using streptozotocin (STZ) shows to be the most popularly used procedure in induction of diabetes in experimental animals. Numerous studies have shown that development of diabetic animal models using certain dosage of STZ on Wistar and Sprague Dawley rats. The aim of the present investigation was conducted to develop type 2 diabetic model using double dose of nicotinamide (NA) and STZ on Sprague Dawley rats within 7 days. Male Sprague-Dawley (250–280 g) were injected with NA, 15 minutes prior the injection of STZ via single and double dose of intraperitoneal (i.p) injection, after overnight fasting. The blood glucose level was monitored from the diabetic animal on day 3, 7, 14 and 21 after the induction of diabetes. Blood glucose levels > 11.0 mmol/L were considered as diabetic condition. In addition, physiological parameters such as food and fluid intakes, changes in body weight and biochemical parameters, blood glucose level were compared with diabetic and control group. In conclusion, the chemically induced diabetic model in Sprague Dawley rats appears to be not suitable compared to the other experimental model which using high fat diet (HFD) and low dose of streptozotocin.

Keywords: Streptozotocin-nicotinamide Induced Diabetes, Sprague Dawley, Type 2 Diabetes.

1. Introduction

Diabetes mellitus (DM) is an endocrine metabolic disorder with multiple aetiology factors which is characterized by high blood glucose concentration due to the defects of insulin secretion and insulin action or both [1]. In 2012, International Diabetic Federation reported that about 366 million people have diabetes in 2011 and this number is expected to be elevated 552 million in 2030. Based on epidemiological studies, among the 2 major types of diabetes, type 2 diabetes is the most common form of diabetes constituting 90–95% of the diabetic population [2]. With the increasing prevalence of type 2 diabetes, the identification of preventative actions has become crucial, requiring the development of effective pre-clinical models for studying possible drug discovery approaches for both diabetic prevention and treatment.

Type 2 diabetes mellitus (T2DM) is a long-term metabolic disorder and it occurred slowly over time and mostly people who are diagnosed with this form of diabetes are obese/overweight. T2DM is characterized by a progressive decline in the effectiveness of insulin action, followed by the incompetence of β-cells to compensate for insulin resistance [3]. Some individuals diagnosed with T2DM have normal
insulin action but distinctly impaired insulin secretion. The β-cells normally recompense insulin resistance by secreting more amounts of insulin to sustain the glucose homeostasis. However, in the course of time, this β-cells function gets impaired leading to deterioration in glucose homeostasis and following development of impaired glucose tolerance and frank diabetes [4]. The risk of developing T2DM increases with age, obesity and lack of physical activity etc. In most cases, people with T2DM normally have a family history with the same disease or other medical problems which are associated with diabetes, such as high cholesterol levels, high blood pressure, or obesity. It is usually controlled with proper diet, weight loss, exercise, as well as oral medications.

Several methods have been used to induce type 2 diabetes mellitus in laboratory animals with variable success and many difficulties. The most commonly used animal models of T2DM include the spontaneous or genetically derived animal models available such as Zucker diabetic fatty rat (ZDF) and as well as Goto-Kakizaki rat (GK), the Otsuka Long Evans Tokushima fatty (OLETF) rat and db/db mouse, which exhibit obesity characteristic, insulin resistance and impaired beta cell function. Even though these animal models have contributed significantly to the understanding of the treatment of T2DM and its complications, these animals are costly and are not easily available for the investigative purposes [4].

Alternative way to develop diabetes is by using the combination of high fat diet and streptozotocin (STZ) or alloxan. Streptozotocin and alloxan are the most well-known diabetogenic chemicals in diabetes research and has been commonly used to induce diabetes in animal model. Although their cytotoxicity is achieved via different pathways, their mechanisms of β-cell and selective action are identical. STZ causes the β-cell toxicity through deoxyribonucleic acid (DNA) alkylation whereas in the case of alloxan, the action is through reactive oxygen species (ROS). Due to its chemical properties, streptozotocin is the agent of choice to develop a diabetic metabolic state in experimental animals as it particularly has greater stability while alloxan (NA). NA, a water-soluble component of the vitamin B complex group, is a biochemical precursor of nicotinamide adenine dinucleotide (NAD). Many studies have shown that NA can improve the energy status in ischemic tissues [8], exhibit antioxidant properties and metabolic improvements [9, 10] as well as inhibit apoptosis [11]. NA has been widely used together with the STZ to induce T2DM within 3 to 7 days after single intraperitoneal (i.p) on animal model, mostly Wistar rats. Based on the previous findings, this study aimed to develop type 2 diabetes using nicotinamide and STZ on Sprague Dawley rats within 3 days.

2. Materials and Methods

2.1 Animals

Healthy adult male Sprague-Dawley rats between 8–9 weeks of age and weighing about 250–280 g were used for the study. The animals were kept under standard laboratory conditions in polypropylene cages at the animal house of the Faculty of Medicine and Health Sciences, Universiti Putra Malaysia, and being maintained under standard conditions (12 h light: 12 h dark cycle; 25 ± 0°C; 35–60% humidity) and they were fed with standard rat pellet diet and water. All animal procedures were approved (UPM/FPSK/PADS/BR-UUH/00410) by Institutional Animal Care and Use Committee (IACUC), FMHS, Universiti Putra Malaysia (UPM), Malaysia.

2.2 Induction of Type 2 Diabetes

Rats were randomly divided into 3 groups (n=6). STZ (Calbiochem, USA) which was dissolved in cold citrate buffer pH 4.5 while NA (Sigma Aldrich, Germany) was prepared by dissolving in 0.9% normal saline. The first group did not receive any injection of NA and STZ and served as control group. The animals in second group received 200 mg/kg NA and 60 mg/kg STZ whereas for the third group were given 150 mg/kg NA and 60 mg/kg STZ via i.p injection. For the second and third groups of animal, the induction procedure with same dose was repeated the next day after the first day of injection with appropriate induction dose. The glucose level was evaluated on day 3, 5 and 7. Diabetes was confirmed by the elevated glucose level in the blood from the tail vein. Non-fasting rats with glucose level > 11 mmol/L were considered as diabetes and the same animals were used for further studies.
2.3 Physiological Parameters
During the experimental period, the animal’s body weight, food and fluid intake were monitored throughout experimental periods, 21 days for each experimental groups.

2.4 Biochemical Parameter
Blood glucose levels of experimental animals were measured using glucometer (Roche, Germany) by taking 0.6µL blood from tail vain onto the test strip on day 0, 3, 7, 14 and 21.

2.5 Histological Analysis
Pancreatic tissue samples were fixed in 10% formalin (Sigma Aldrich, Germany) solution, embedded in paraffin, cut into a tissue section of 5 μm thickness and stained with hematoxylin-eosin (H&E). Slides were then observed under light microscope for histopathological analysis.

2.6 Statistical Analysis
Results are expressed in mean ± S.D. Data were analyzed using one-way analysis of variance and Duncan’s multiple range tests. Statistical analyses were performed using Graphpad Prism. P value ≤ 0.05 was considered as statistically significant.

3. Results
3.1 Physiological and Biochemical Parameters
The results illustrated in figure 4 show that animals in group 2 and 3, which received double doses of NA and STZ, effectively induced hyperglycemic condition. Fasting blood glucose levels before NA and STZ injection were 5.2 mmol/L and 4.96 mmol/L, respectively. Within 3 days of post injection, significant increment in the blood glucose level was observed as compared to control group. The level of glucose was higher than 11 mmol/L, indicated that the animal was in diabetic state. The administration of higher dose of STZ with lower dose of NA showed a significant effect on blood glucose levels compared to the lower dose.

Body weight of normal and diabetic rats is shown in the figure 1. The rats in hyperglycemic condition exhibited a tendency to increase in fluid intake and reduce in food intake compared to normal rats as shown in figure 3 and 4. The rats appear to urinate a lot compared to normal rats and this explains the decrease of body weight in diabetic rats.

3.2 Histological Examination of Pancreas
Figure 5 represent islets of langerhans from a normal (Figure 5A) and STZ-induced diabetic rats (Figure 5B and 5C), respectively. Comparison of these 3 figures clearly indicates that reduction in the number of pancreatic islets...
as well as their number of β-cells in the diabetic rats compared to the normal rats. As it is evident from Figure 5B and 5C, the islets are unevenly shaped and rather small and atrophic in contrast to the islets in Figure 5A.

### 4. Discussion

Diabetes mellitus (DM) is a metabolic disorder resulting from a deficiency in insulin secretion, insulin action, or both [12]. Impaired in insulin secretion and the development of insulin resistance contribute to the etiology of type 2 diabetes [13]. The reduction of insulin secretion because of functional defect and the loss of surviving pancreatic β-cells lead to hyperglycemia and subsequent decline in insulin sensitivity [14].

Clinical experience demonstrates that type 2 diabetes can also be present in the absence of an obese phenotype [14]. A new experimental diabetic model in adults rats by administering STZ and partially protected it with a suitable dose of NA has been described by Masielo et al. in 1998 using Wistar rats. The study demonstrated that this syndrome shares a number of characteristics with human type 2 diabetes and is verified by moderate stable hyperglycemia, glucose intolerance, altered but significant glucose-stimulated insulin secretion, in vivo and in vitro [15].

STZ has been widely used to induce T1DM and T2DM in animal models especially rats and mice when it was administered either intravenously or intraperitoneally [16]. A significant example of endogenous chronic oxidative stress due to the resulting hyperglycemia was shown in
STZ induced diabetes model [17]. STZ is a pancreatic cell chemical toxin that induces prompt and irreversible necrosis of pancreatic cells [16]. Previous investigations showed that different strains of rats have been reported to respond differently to STZ injection using doses ranging from 25 to 100 mg/kg [17]. Administration of STZ and NA result in a partial loss of β-cell mass by necrosis and/or apoptosis [18]. STZ-induced diabetic rats demonstrated severe hyperglycemia associated with a depletion of pancreatic insulin content/action [19]. Therefore, NA was used to prevent excessive pancreatic injury induced by STZ.

This study was initiated with the objective of developing an ideal model for type 2 diabetes in animal model that would mimics the metabolic characteristics of human type 2 diabetes. Additionally, it should be less expensive, simply available and taking fairly a shorter period to develop diabetes.

When evaluating the results of this study, it was seen that the dosages of STZ which are 60 and 65mg/kg have effectively establish hyperglycemic condition in rats. However, higher dose of STZ result in very high glucose level within 3 days. Animals in group 2 and 3 could not tolerate the elevated amount of glucose more than 14 days, following the given dosage of STZ. Extremely high glucose concentration result making it toxic to the body and cause the death of animals. NA did not fully protect the pancreatic β-cells and result in the elevated amount of glucose in blood due to the destruction of β-cells which causing very less production and action of insulin.

Almost 70% of islet cells are formed by beta cells which are responsible for producing insulin. The pancreas of diabetic rats demonstrated degeneration, necrosis and destruction of beta cells in the islets of Langerhans. Depletion of beta cells will therefore result in insulin deficiency which will contribute to a metabolic disorder in carbohydrate, protein and fat metabolism following high glucose concentration [20]. In this present study, higher concentration of blood glucose in STZ-NA induced diabetic rats showed the diabetes condition was successfully achieved and this was supported by the histological finding of the pancreatic tissues which demonstrated remarkable tissue destruction with a very low count of beta cells in islets of langerhans that could be observed in rats of the diabetic groups.

5. Conclusion

Findings from this investigation suggest that double injection of NA and STZ demonstrated effective way to induce type 2 diabetes in this strain of rats; however this dosage of STZ and NA was not suitable for a longer period of experimental study. Hence, we suggest that researchers may select different strain of rats rather than Sprague Dawley rats for induction type 2 diabetes.

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7. References