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Research article

A murine model of type 2 diabetes mellitus developed using a combination of high fat diet and multiple low doses of streptozotocin treatment mimics the metabolic characteristics of type 2 diabetes mellitus in humans

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ABSTRACT

Introduction: A murine model of type 2 diabetes mellitus was used to compare the antidiabetic effects of the dipeptidyl peptidase-4 (DPP4) inhibitor vildagliptin and biguanide, metformin.
Methods: Swiss albino mice (n = 20 males; n = 25 females) were given high fat diet (HFD) ad libitum for 3 weeks followed by low dose (40 mg kg^{-1} body weight, bw daily) of streptozotocin (STZ) intraperitoneally five times from the 22nd day of treatment onwards, with HFD continued up to 26th day. Controls (n = 15 males; n = 15 females) were fed normal balanced diet without administration of STZ. Successful induction of diabetes mellitus was confirmed by testing for fasting blood glucose, intraperitoneal glucose tolerance and intraperitoneal insulin sensitivity. Diabetic mice were administered vildagliptin (10 mg kg^{-1} bw daily) and metformin (50 mg kg^{-1} bw daily) orally for 4 weeks. Control, diabetic, vildagliptin and metformin-treated diabetic mice were evaluated for alterations in lipid profile using blood serum and histopathology and oxidative stress using tissues including liver, kidney and heart.
Results: Diabetic mice showed significant alterations in lipid profile, tissue histopathology, impaired glucose tolerance, lower insulin sensitivity and elevated lipid peroxidation and protein carbonylation, with depressed catalase activity, when compared to age and gender-matched controls. Metformin and vildagliptin ameliorated the abovementioned diabetic conditions, with vildagliptin found to be more effective.
Discussion: A murine model developed by the combination of HFD and multiple low dose of STZ mimics the metabolic characteristics of type 2 diabetes mellitus in humans, and may be useful for antidiabetic drug screening.

1. Introduction

Type 2 diabetes mellitus (T2DM) is a heterogeneous, multifactorial disorder characterized by hyperglycemia and gradual decline in insulin action (insulin resistance), followed by the inability of beta (β)-cells to compensate for insulin resistance (pancreatic β-cell dysfunction) (Srinivasan, Viswanad, Asrat, Kaul, & Ramarao, 2005). In the long run, T2DM often gives rise to severe complications of macrovascular origin such as cardiovascular diseases, peripheral vascular diseases and stroke or of microvascular origin such as retinopathy and nephropathy (Cade, 2008). T2DM accounts for over 95% of all diabetes cases, and it is estimated that by the year 2030 the number of individuals with T2DM will rise to 552 million (Ryden et al., 2013). This alarming scenario has fostered research into the development of novel pharmacologic agents for the treatment of T2DM, as well as evaluation of the safety and efficacy of old as well as new anti-diabetic medication (Inzucchi et al., 2012).

The incretin hormone GLP-1 is secreted by the enteroendocrine L-cells in response to fatty meals and carbohydrates, and serves to stimulate insulin secretion (Kieffer & Habener, 1999). However, GLP-1 is rapidly degraded by the enzyme dipeptidyl peptidase-4 (DPP-4) (Freeman, 2009). Recently, it was demonstrated that DPP-4 inhibitors could preserve β-cell mass (Akarte, Srinivasan, & Gandhi, 2012), mainly through increasing β-cell proliferation and protecting β-cells from apoptosis (Takeda et al., 2012). Metformin, a biguanide, is a widely used antidiabetic drug for the treatment of T2DM which primarily works by lowering the ATP/AMP ratio and activating the LKB1/AMP kinase (AMPK) pathway (Hardie, 2006). This leads to the inhibition of gluconeogenesis in hepatocytes (Shaw et al., 2005) resulting in decreased hepatic glucose output and a secondary decline in insulin levels.

Animal models mimicking human T2DM are pivotal for the biological screening of anti-diabetic drugs, and several approaches have been

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used to develop such models. These include genetic manipulation such as the *ob*/*ob* mouse, *db*/*db* mouse, Zucker *fa*/*fa* rat etc. and various treatment regimens with one or more chemicals such as alloxan, streptozotocin etc. (Chen & Wang, 2005; Hu et al., 2013; Omabe, Nwudele, Omabe, & Okorocha, 2014; Ozcan et al., 2004; Tartaglia et al., 1995; Y. Zhang et al., 1994; Zucker, 1965). Regardless of many animal models, both genetic and chemical, available for T2DM, most of them do not simulate the clinical situation of human T2DM because of large heterogeneity in humans. Furthermore, these animals are not easily available and tend to be expensive.

Individuals diagnosed with T2DM are found to be obese with delayed or insufficient insulin secretion relative to glucose load, and are incapable of responding to insulin in peripheral tissues (Arulmozhi, Kurian, Bodhankar, & Veeranjaneyulu, 2008). Thus, β cells of the pancreas are incapable of maintaining normal glucose tolerance as a result of which glucose concentration in the plasma increases (Kahn, Cooper, & Del Prato, 2014). A good way of initiating the insulin resistance associated with T2DM in humans is the administration of high fat diet (HFD) to animals, thus inducing obesity, which acts as a known risk factor for T2DM. In addition, a low dose of the β-cell toxin streptozotocin (STZ) is commonly used to hasten the progress of T2DM in mice similar to the condition observed in human T2DM. High doses of STZ is toxic to the insulin producing β-cells of pancreatic islets and impairs insulin secretion by inducing β-cell death through alkylation of DNA, thus mimicking type 1 diabetes mellitus, while low-doses of STZ cause a mild impairment of insulin secretion attributed to the later stage of T2DM (Reed et al., 2000; Srinivasan et al., 2005; Szkudelski, 2001). Overall, these two stressful conditions imitate in animals, the development of T2DM found in the human condition, although in a shorter duration.

In addition to hyperglycemia, systemic or local elevations in insulin may contribute to aberrant lipid metabolism and vascular wall function (Kunjathoor, Wilson, & LeBoeuf, 1996). Efforts have therefore been made to develop rodent models of T2DM induced by HFD followed by low-dose STZ. The multiple low-dose STZ injections cause a steady, autoimmune destruction of β-cells instead of the rapid destruction stimulated by a single high-dose STZ injection (Kannan, Tokunaga, Moriyama, Kinoshita, & Nakamura, 2004; McEvoy, Andersson, Sandler, & Hellerstrom, 1984), which could effectively be used along with HFD treatment to mimic the pathological condition of human T2DM. These models closely imitate the natural history of the progression of T2DM, from insulin resistance to β-cell dysfunction, as well as the associated metabolic characteristics of human T2DM such as abnormalities in lipid metabolism (Li, Ji, Zhong, Lin, & Lv, 2015; Reed et al., 2000; Srinivasan et al., 2005). However, there are inconsistencies pertaining to the administered dose of STZ and the methodology adopted in these studies. Moreover, in the study by Arulmozhi et al. (2008), only male Swiss albino mice of LACA strain were used to observe the effects of various anti-diabetics and antihyperlipidemics on total cholesterol and triglyceride levels. To the best of our knowledge, only one study has reported the development of a T2DM model in both male and female mice so far with only the males exhibiting glucose intolerance and hyperglycemia (Kim & Saxton, 2012). The rationale of the present study is to develop an appropriate, cost effective and stable animal model analogous to human T2DM through a combination of HFD with multiple low-dose STZ injections. In order to improve over previously reported models, we aimed to develop a suitable murine model in both male and female mice that exhibits concomitantly elevated oxidative stress, altered tissue histopathology, and altered carbohydrate and lipid metabolism associated with T2DM in humans, not reported in previous models.

We also aimed to verify whether the model developed could be used for the screening of anti-diabetic drugs. We therefore studied the efficacy of the dipeptidyl peptidase-4 (DPP-4) inhibitor vildagliptin (Akarte et al., 2012) vis-à-vis metformin in our model. To the best of our knowledge, this is the first study into the efficacy of vildagliptin in HFD-multiple low dose of STZ-induced T2DM in mice, which has been evaluated on the basis of the parameters of glucose metabolism, lipid profile (Srinivasan et al., 2005) and biochemical markers of oxidative stress (Tiwari, Pandey, Abidi, & Rizvi, 2013). The study was conducted in both male and female mice to reveal gender specific changes, if any.

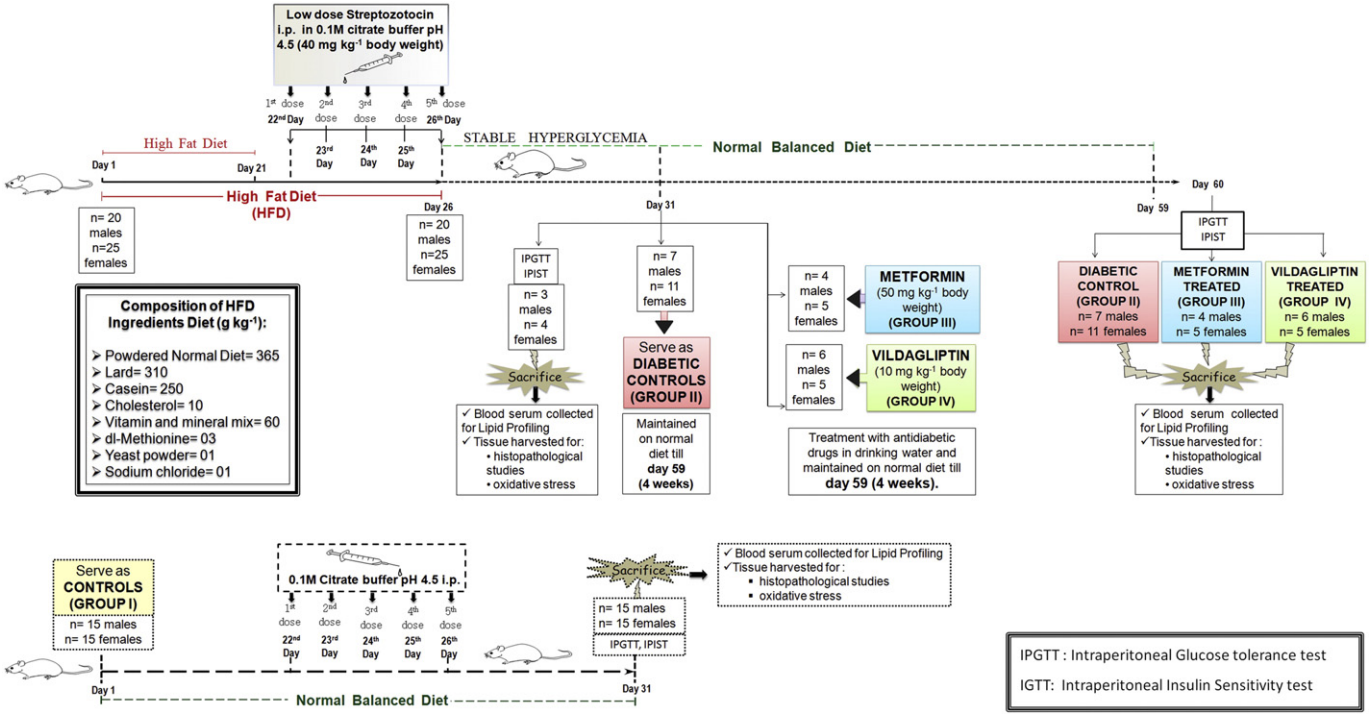

Fig. 1. Schematic representation of the study design.