Development of a type II diabetic mellitus animal model using Micropig®

Myeong-Seop Lee^{1#}, Ki-Duk Song^{2#}, Hee-Jun Yang¹, Chester D. Solis¹,
Soo-Hyeon Kim³, Woon-Kyu Lee^{4,5*}

¹Medikinetics Co., Ltd., Pyeong-Taek, Korea

²Center for Agricultural Biomaterials, Seoul National University, Seoul, Korea

³Qu-BEST Consulting Co., Ltd., Seongnam, Korea

⁴Laboratory of Developmental Genetics,

⁵Center for Advanced Medical Education by the BK21 Project, College of Medicine, Inha University, Incheon, Korea

Diabetes, which has shown an explosive increase in terms of its incidence, is regarded as a serious disease that must be overcome for the sake of human life. Among animal models used for testing of drug efficacy, the mini-pig model has shown a rapid uptake due to its many similarities with human, particularly concerning the pharmacokinetics of compounds after subcutaneous administration, the structure and function of the gastrointestinal tract, the morphology of the pancreas, and overall metabolic status. Based on these various advantages, we sought to develop an animal model of type II diabetic mellitus using the Micro-pig, which differs from other miniature pigs. We used six male Micro-pigs for induction of a moderate insulin deficient model with nicotinamide (NIA)/streptozotocin (STZ) treatment and three animals for control. For evaluation of incidence of type II diabetes, we measured blood glucose level, and performed oral glucose tolerance test and immunohistochemistry on pancreatic tissue using insulin antibody. Compared to control animals, all animals treated with NIA/STZ showed high levels of glucose and low levels of insulin. In addition, we observed the partially destroyed beta cell population from tissue of the pancreas in treated animals. Based on these results, we report that the Micro-pig model developed in this study can be used for testing of the efficacy of therapeutic agents for treatment of Type 2 diabetic mellitus.

Key words: Type 2 diabetic mellitus, Micropig, glucose level, NIA, STZ

Received 23 April 2012; Revised version received 29 August 2012; Accepted 30 August 2012

Diabetes mellitus (DM) encompasses a heterogeneous group of disorders with the common characteristic of elevated blood glucose, i.e., Hyperglycemia, due to disruption in metabolism of carbohydrates, fat, and protein, resulting from impaired insulin secretion [1,2,3]. Based on etiology, DM can be divided into four groups, including type 1 DM, type 2 DM, other specific types of diabetes, and gestational diabetes [4]. Among them, type 2 DM is characterized by relative moderate insulin

deficiency resulting from a decreased effect of insulin in target tissues, e.g., muscles and adipose tissue (insulin resistance), or a secretory defect of insulin with or without insulin resistance [4,5].

Use of appropriate animal models for type 2 DM is important for understanding its pathogenesis and development and testing of therapeutics for treatment of this disease. Rodent models provide a number of advantages over other mammals; however, there are also

[#]These authors contributed equally to this work.

*Corresponding author: Woon-Kyu Lee, Laboratory of Developmental Genetics, College of Medicine, Inha University, 100 Inharo, Nam-gu, Incheon 400-712, Korea
Tel: +82-32-890-1182; Fax: +82-32-890-1199; E-mail: wkleee@inha.ac.kr

This is an Open Access article distributed under the terms of the Creative Commons Attribution Non-Commercial License (<http://creativecommons.org/licenses/by-nc/3.0>) which permits unrestricted non-commercial use, distribution, and reproduction in any medium, provided the original work is properly cited.

several drawbacks, which might primarily include the fact that, genetically and physiologically, rodents are not as close to humans, compared to other mammals, e.g., monkey and pig, etc. Among them, pigs share the structure and function of the gastrointestinal tract, the development and morphology of the pancreas, and overall metabolic status with humans [6,7,8], and have also demonstrated comparable pharmacokinetics of the compound via subcutaneous administration [9] with humans; therefore, use of pigs might be a good alternative for replacement of rodents for use in diabetes research. Owing to their small size and ease of handling, even at full maturity, mini-pigs were found to be suitable for use in long-term studies. The pancreas of mini-pigs resembles the human pancreas in size, shape, and position. In addition, the amino acid sequences of insulin between pig and human differ only in a single residue. Based on these various advantages, the mini-pig is of particular interest for studies related to human type 1 DM [10].

High fat and cholesterol (HFC) diets with or without streptozocin (STZ) have been adapted for the establishment of animal models for type 2 DM, such as pig [7,11,12] and rat [13]. STZ is a chemical that is particularly toxic to insulin-producing pancreatic β -cells in mammals. It is used in medical research for the construction of animal models for type 1 DM [14,15]. In addition, treatment with STZ with nicotinamide (NIA), which modulates STZ-mediated β -cell destruction and reduces its severity, has been widely used for induction of type 2 DM in animal models, including mouse [16], rat [17,18], hamster [19], and Göttingen minipig [9]. However, variability in the establishment of STZ-mediated DM, including both type 1 DM and type 2 DM, has restricted development of a general protocol using STZ alone as well as STZ with NIA [20,21]. Therefore, testing and optimization of procedures for induction of type 2 DM for the new line of animals is worthwhile. In this study, in order to establish a useful animal model for diabetes research, we performed the induction of type 2 DM in Micro-pigs, which differ from Göttingen minipigs [22]. The Micro-pig is a breed of laboratory swine developed and maintained until 2005 by the Medi kinetics Co (MK) breeding program for biomedical research and nonclinical trials.

All animals included in the study were adult male Micro-pigs over six months of age with less than 20 kg of body weight. Animals were obtained from the barrier unit at Medi Kinetics Co (Pyeongtaek, Korea). All

animals were housed in single pens under controlled conditions (temperature was kept between 18 and 22°C, relative air humidity was 30-70% with 15 air changes/h) with a 12:12-hr light-dark cycle and allowed free access to water. Principles of laboratory animal care were followed in accordance with the Guide for Animal Experimental Protocol (MK-IACUC:2010-0088) and the type of study was approved by the Institutional Animal Care and Use Committee of Medi kinetics Co. The standard feeding regime was applied to the animals used in this study.

Animals were fed a pellet diet sterilized by 2M rad radiation (Purina, Seongnam, Korea) and received sterilized water *ad libitum*. For induction of type 2 DM, NIA and STZ were administered to male Micro-pigs via intravenous (i.v.) injection. NIA (Cat No. N0636) and STZ (Cat No. S0130) were purchased from Sigma-Aldrich Co. (St. Louis, MO). NIA solution was prepared by dissolving in sterile saline solution at a concentration of 300 mg/mL and administered via i.v. within 15 min prior to administration of STZ. Injection of 67 mg/kg of NIA was administered to each animal. STZ was dissolved in sodium citrate buffer at a concentration of 62.5 mg/mL and injected via i.v. for a period of 1-2 minutes. A total of 125 mg/kg STZ was administered per animal. Blood was taken before feeding, following fasting for 12-16 hr in controls or 12-16 hr in NIA/STZ administration groups. Measurement of blood glucose levels was performed weekly before and after induction of DM, using a blood glucose tester (Accu-Chek® Go) (Roche Diagnostics). At the end of the experiments, all Micro-pigs were scarified and subjected to autopsy. Specifically, pancreatic glands were removed and fixed in 10% neutralized buffered formalin, processed, and embedded in paraffin, followed by sectioning, hematoxylin and eosin (H&E) staining, and immunostaining with insulin antibody (Cat No. ab58977, Abcam).

In this study, Micro-pigs were treated with NIA/STZ in order to recapitulate human type 2 DM, which is characterized by moderately elevated levels of glucose and insulin resistance. Compared to controls, Micro-pigs treated with NIA/STZ showed elevated levels of glucose ($P < 0.05$, *t*-test). Elevation of glucose occurred two weeks after administration of NIA/STZ in type 2 DM pigs and the maximum increase was achieved at four weeks after treatment, with the maintenance of a hyperglycemia trend until the end of the experiment (Figure 1). These data provided indication that the NIA/

Figure 1. Blood glucose levels of fasting Micro-pigs was checked before feeding using Accu-Chek® Go. C: Control Micro-pig group (n=3). D: NIA /STZ- treated Micro-pig group (n=3).

Figure 2. Serum insulin levels of fasting Micro-pigs was checked before feeding using ELISA. C: Control Micro-pig group (n=1). D: NIA /STZ- treated Micro-pig group (n=3).

STZ regimes used in this study might be effective for induction and maintenance of type 2 DM in Micro-pigs, which is in agreement with findings from previous reports using Göttingen minipigs. The dose of NIA/STZ used in this study differs from that used in the previous study, reflecting the intrinsic variability of STZ-induced DM. In addition, lower levels of serum insulin were observed in NIA/STZ-treated Micro-pigs ($P < 0.05$, Figure 2), compared to controls, suggesting impaired production and/or insulin from β -islet cells. An insulin antibody was used to perform immunohistochemistry (IHC) in NIA/STZ-induced type 2 DM pancreas. Results of IHC analysis revealed a significant decrease in the positivity of insulin expressing β -cells from NIA/STZ treated pancreas (Figure 3D). In addition, enlarged islets were observed, indicating an inflammatory response to NIA/STZ treatment (Figure 3B). In conclusion, based on the results described above, we suggest that the chemically induced Micro-pig diabetic model can be used for testing of the efficacy of candidate diabetic drugs and products for use in diagnosis and treatment of

Figure 3. Immunohistochemistry (IHC) of pancreas with insulin antibody. A: HE staining of pancreas of control Micro-pig. B: IHC of pancreas of control Micro-pig with insulin Ab. C: HE staining of pancreas of NIA/STZ -treated Micro-pig. D: IHC of pancreas from NIA/STZ-treated Micro-pig with insulin Ab.

patients with diabetes mellitus.

Acknowledgments

This Research was supported in part by the Technology Development Program for Agriculture and Forestry (Project No.110045-2), Ministry for Food, Agriculture, Forestry and Fisheries, Republic of Korea, and a grant from the Next Generation BioGreen 21 program (No. PJ008196), Rural Development Administration, Republic of Korea.

References

- Georg P, Ludvik B. Lipids and Diabetes. *J Clin Basic Cardiol* 2000; 3: 159-162.
- Nyholm B, Pøksen N, Juhl CB, Gravholt CH, Butler PC, Weeke J, Veldhuis JD, Pincus S, Schmitz O. Assessment of insulin secretion in relatives of patients with type 2 (non-insulin-dependent) diabetes mellitus: evidence of early beta-cell dysfunction. *Metabolism* 2000; 49(7): 896-905.
- Harris MI. 2004. Definition and classification of diabetes mellitus and the criteria for diagnosis. In *Diabetes Mellitus: A Fundamental and Clinical Text*, Philadelphia: Lippincott Williams & Wilkins, pp 457-467.
- Sparre T, Larsen MR, Heding PE, Karlsen AE, Jensen ON, Pociot F. Unraveling the pathogenesis of type 1 diabetes with proteomics: present and future directions. *Mol Cell Proteomics* 2005; 4(4): 441-457.
- Bellinger DA, Merricks EP, Nichols TC. Swine models of type 2 diabetes mellitus: insulin resistance, glucose tolerance, and cardiovascular complications. *ILAR J* 2006; 47(3): 243-258.
- Miller ER, Ullrey DE. The pig as a model for human nutrition. *Annu Rev Nutr* 1987; 7:361-382.

7. Barth CA, Pfeuffer M, Scholtissek J. Animal models for the study of lipid metabolism, with particular reference to the Göttingen minipig. *Adv Anim Physiol Anim Nutr* 1990; S20:39-49.
8. Swindle MM, Smith AC: Comparative anatomy and physiology of the pig. *Scand J Lab Anim Sci* 1998; 25:11-21.
9. Larsen MO, Wilken M, Gotfredsen CF, Carr RD, Svendsen O, Rolin B. 2002. Mild streptozotocin diabetes in the Göttingen minipig. A novel model of moderate insulin deficiency and diabetes. *Am J Physiol Endocrinol Metab* 2002; 282(6): E1342-1351.
10. Lee PY, Park SG, Kim EY, Lee MS, Chung SJ, Lee SC, Yu DY, Bae KH. Proteomic analysis of pancreata from mini-pigs treated with streptozotocin as a type I diabetes models. *J Microbiol Biotechnol* 2010; 20(4):817-820.
11. Koopmans SJ, Dekker R, Ackermans MT, Sauerwein HP, Serlie MJ, van Beusekom HM, van den Heuvel M, van der Giessen WJ. Dietary saturated fat/cholesterol, but not unsaturated fat or starch, induces C-reactive protein associated early atherosclerosis and ectopic fat deposition in diabetic pigs. *Cardiovasc Diabetol* 2011; 10: 64.
12. Koopmans SJ, Mroz Z, Dekker R, Corbijn H, Ackermans M, Sauerwein H. Association of insulin resistance with hyperglycemia in streptozotocin-diabetic pigs: effects of metformin at isoenergetic feeding in a type 2-like diabetic pig model. *Metabolism* 2006; 55(7): 960-971.
13. Pascoe WS, Storlien LH. Inducement by fat feeding of basal hyperglycemia in rats with abnormal beta-cell function. Model for study of etiology and pathogenesis of NIDDM. *Diabetes* 1990; 39(2): 226-233.
14. Herr RR, Jahnke JK, Argoudelis AD. The structure of streptozotocin. *J Am Chem Soc* 1967; 89(18): 4808-4809.
15. Yamamoto H, Uchigata Y, Okamoto H. Streptozotocin and alloxan induce DNA strand breaks and poly(ADP-ribose) synthetase in pancreatic islets. *Nature* 1981; 294(5838): 284-286.
16. Schein PS, Cooney DA, McMenamin MG, Anderson T. Streptozotocin diabetes--further studies on the mechanism of depression of nicotinamide adenine dinucleotide concentrations in mouse pancreatic islets and liver. *Biochem Pharmacol* 1973; 22(20): 2625-2631.
17. Masiello P, Bergamini E. Nicotinamide and streptozotocin diabetes in the rat. Factors influencing the effectiveness of the protection. *Experientia* 1977; 33(9): 1246-1247.
18. Masiello P, Broca C, Gross R, Roye M, Manteghetti M, Hillaire-Buys D, Novelli M, Ribes G. Experimental NIDDM: development of a new model in adult rats administered streptozotocin and nicotinamide. *Diabetes* 1998; 47(2): 224-229.
19. Farah KM, Atoji Y, Shimizu Y, Takewaki T. Isulinotropic properties of Nigella sativa oil in Streptozotocin plus Nicotinamide diabetic hamster. *Res Vet Sci* 2002; 73 (3): 279-282.
20. Engle MJ, Perelman RH, McMahon KE, Langan SM, Farrell PM. Relationship between the severity of experimental diabetes and altered lung phospholipid metabolism. *Proc Soc Exp Biol Med* 1984; 176(3): 261-267.
21. Rodrigues B, Poucheret P, Battel ML, McNeill JH. Streptozotocin-induced diabetes: induction, mechanism(s) and dose dependency. In: *Experimental Models of Diabetes*, edited by McNeill JH. Boca Raton, FL: CRC. 1999; pp 3-19.
22. Larsen MO, Rolin B, Wilken M, Carr RD, Gotfredsen CF. Measurements of insulin secretory capacity and glucose tolerance to predict pancreatic beta-cell mass in vivo in the nicotinamide/streptozotocin Göttingen minipig, a model of moderate insulin deficiency and diabetes. *Diabetes* 2003; 52(1): 118-123.