InterConf
Scientific Publishing Center

THE ISSUE CONTAINS:

Proceedings of the 4th International Scientific and Practical Conference

SCIENTIFIC PROGRESSIVE METHODS AND TOOLS

Riga, Latvia
6-8.10.2024

Scientific Collection
InterConf

№ 219
October, 2024

OPEN ACCESS

InterConf
Scientific Publishing Center

Scientific Collection «InterConf»

№ 219
October, 2024

THE ISSUE CONTAINS:

Proceedings of the 4th International
Scientific and Practical Conference

SCIENTIFIC PROGRESSIVE METHODS AND TOOLS

RIGA, LATVIA
October 6-8, 2024

RIGA
2024

Proceedings of the 4th International Scientific and Practical Conference «Scientific Progressive Methods and Tools»
(October 6-8, 2024). Riga, Latvia

No 219

BIOLOGY AND BIOTECHNOLOGY

Competitive estimation of the validity of different animal models of diabetes mellitus

Horkunenko Oksana O.[1], Yezhel Iryna M.[2], Yanitska Lesya V.[3]

[1] PhD, Associate professor, senior teacher of medical biochemistry and molecular biology department *Bogomolets National Medical University;* Ukraine

[2] PhD, assistant teacher of medical biochemistry and molecular biology department; *Bogomolets National Medical University;* Ukraine

[3] PhD, Associate professor, Head of medical biochemistry and molecular biology department; *Bogomolets National Medical University;* Ukraine

Abstract. Experimental animal models of diabetes mellitus are widely used to study the pathogenesis of disease, the therapeutic effects of medications, their safety and effectiveness. In this paper the different types of rodent models of type 2 diabetes mellitus were analyzed aiming to estimate their advantages and limitations. The understanding of the features of each experimental model is critical for the proper development of experimental design and correct interpretation of experimental data.
Keywords: *diabetes mellitus, animal models, rodents.*

Diabetes mellitus (DM) is a globally widespread disease associated with insufficient or absent insulin secretion or with development of insulin resistance. A characteristic feature of DM is chronic hyperglycemia, which is the main cause of development of diabetic complications, in particular cardiovascular diseases, diabetic retinopathy, nephropathy, etc.

To fight the diabetic complications, the scientists around the globe investigate the therapeutic effect of drugs with animals. The animal models allow to study *in vivo* the biochemical mechanisms of action of medications and to check their safety and effectiveness. Different animals can be used as models to study the mechanisms of development of diabetes and its complications. The examples are domestic cats, which have similar to humans living conditions and exogenous risk factors (obesity, insufficient physical activity); pigs,

which have similar anatomical and morphological features of cardiovascular system, gastrointestinal tract and mechanisms of metabolic processes; primates, due to the similar mechanisms of development of diabetic complications [1, 2]. However, in many animal models of experimental type 1 and type 2 DM, the rodents are used due to their small size, simple housing, and cost effectiveness. Experimental animal models of DM, most often the rodent models, allow to study in detail the pathogenesis of disease, the mechanisms and risk factors for development of diabetic complications and to estimate the effectiveness of approaches of its treatment.

Classification of animal models of DM is based on the method of diabetes induction and on the type of diabetes; for T2DM the animal models with or without obesity are distinguished. Considering the pathobiochemical features of type 1 and type 2 diabetes, the valid experimental models should be adapted to each condition taking into account the mechanism of diabetes development and expected complications. The aim of this paper is to consider the rodent models of T2DM, to make their evaluation and critical analysis.

Development of T2DM in animals can be induced by surgical, chemical or other methods that lead to appearance of characteristic clinical signs of disease, such as reduced insulin secretion, insulin resistance, hypoglycemia, etc.

Surgical methods of induction of DM. Historically, one of the first studies related to the role of insulin in pathogenesis of T1DM was the removal of pancreas in a dog, which led to development of polyuria [3]. Partial pancreatectomy in experimental animals, in particular in rodents, do not cause the development of severe forms of diabetes and is manifested by mild to moderate hyperglycemia depending on the percentage of pancreas removed [1, 4]. The combination of partial pancreatectomy with chemical agents such as alloxan or streptozotocin (STZ) leads to development of stable hyperglycemia and diabetes [5]. In their study Kurub and Bhonde [6] indicated that removal of 50% of the pancreas with further injection of STZ and nicotinamide to mice led to persistent hyperglycemia and appearance of the signs of insulin-independent DM. Partial pancreatectomy has obvious limitations related to its invasiveness and the risk of post-operative complications, often it is used to study regeneration of the pancreas and transplantation of insulin-producing cells [7, 8].

Chemically induced DM. The well-known chemical agents

that induce DM at parenteral injection are STZ and alloxan, which manifest β-cell toxicity and cause hyperglycemia within days of administration [5]. The dosage of drug required for induction of diabetes is determined by the animal type, by the protocol of drug administration, and by nutritional status of the animal [9]. The drug is usually administered in 5-7 days before the experiment to provide stable hyperglycemia [10]. In addition to alloxan and STZ the other agents such as cyclophosphamide, dithizone can be used to induce diabetes.

The alloxan-induced diabetes is a form of insulin-dependent diabetes, which pathogenesis is related to selective inhibition of insulin secretion by β-cells and to formation of reactive oxygen species (ROS) that leads to destruction of β-cells of the pancreas. Alloxan is transported to the cells by facilitated diffusion by GLUT-2 because the spatial structure of alloxan is similar to glucose. The influence of alloxan on insulin secretion is related to its ability to oxidize two thiol -SH groups in the active center of glucokinase inactivating it. Generation of ROS is related to the cyclic redox process in which alloxan in the presence of intracellular thiols is reduced to dialuric acid; its further re-oxidation leads to formation of alloxan radical and to generation of superoxide radical in the presence of oxygen [11]. Superoxide radical is reduced to hydrogen peroxide. The low activity of catalase in the β-cells causes formation of hydroxyl radicals which realize the cytotoxic effect of alloxan [12].

Important limitation of the model of alloxan-induced diabetes is the insufficient stability of hyperglycemia and its diabetogenic and toxic effects on animals. Also, in opinion of some authors, alloxan cannot be used to induce T2DM [9, 13], which accounts in about 90-95% of all cases of DM in humans.

Streptozotocin (STZ), same as alloxan, is a cytotoxic glucose analogue, which is absorbed by the β-cells of the pancreas causing their destruction. The transport of STZ to the β-cells occurs by facilitated diffusion by GLUT-2. The toxic action of STZ is related to alkylation of nitrogenous bases in DNA, which causes its damage [14]. Reparation of DNA structure by poly(ADP-ribose)-polymerase (PARP) leads to depletion of the cellular pool of NAD^+ and ATP [12] and to necrosis of the β-cells. The ability of STZ to suppress the glucose-induced secretion of insulin is associated with the damage of mitochondrial DNA and disordered signaling

function. To a certain extent the cytotoxicity of STZ is related to its ability to induce ROS and nitrogen (II) oxide that leads to development of oxidative/nitrosative stress and inflammation [14].

Streptozotocin is used for induction of both T1DM and T2DM. Single injection of a high dose of STZ (up to 200 mg/kg) or multiple administration of the moderate doses (40-70 mg/kg) leads to destruction of the β-cells and to development of T1DM [15], while low dose injection of STZ (25 mg/kg) causes the lower damage to the β-cells leading to T2DM [16]. Administration of nicotinamide partially protects β-cells of the pancreas from STZ-mediated cytotoxicity [13] and leads to development of insulin deficient T2DM, which is characterized by moderate hyperglycemia related to the partial loss of the β-cell function [15]. Administration of the low dose of STZ in combination with the high-fat diet leads to development of insulin resistance and progressive β-cell dysfunction, which is typical for T2DM [17]. Streptozotocin has many advantages over alloxan for induction of DM because of its larger selectivity to the β-cells, its ability to induce both types of DM, fewer adverse effects and lower mortality of the animals [13].

The models of chemically induced DM are simple, have high reproducibility and can be used for both types of DM. However, the alloxan and STZ may cause development of unwanted complications from organs and tissues that express GLUT-2 (liver and renal tubules), which makes difficulties in proper interpretation of results and their extrapolation to the manifestations of T2DM. Furthermore, at induction of T2DM, streptozotocin does not mimic the influence of epigenetic factors such as obesity, which play an important role in development of T2DM in humans [18].

Spontaneous DM. Due to the high heterogeneity of manifestations of T2DM, there are many rodent models which provide the possibility to study the complex pathogenesis and pathobiochemical process in type 2 diabetes. The models of spontaneous T2DM are considered as most useful to study the mechanisms of development of insulin resistance in humans.

Obesity is one of the key risk factors for development of T2DM, as it may lead to reduced insulin secretion and to insulin resistance. Examples of spontaneous T2DM obese models are ob/ob and db/db mice and Zucker rats. [19].

The ob/ob mice have a point mutation in leptin gene, which leads to development of obesity, hyperinsulinemia, and

insulin resistance at the age of 4 weeks. At a young age, they show hyperphagia, hyperglycemia, and glycosuria without developing ketonemia [5], thus modeling the certain aspects of T2DM. Manifestations of diabetes get decreased with age, which is seen in normalization of insulin level in the blood plasma and improvement of insulin resistance. The limitation of this model is inability to fully imitate the diabetic state in humans because most of the typical complications of DM do not develop in ob/ob mice [19].

The db/db mice have a point mutation in leptin receptor gene (LepR). They demonstrate hyperphagia, obesity, hyperinsulinemia and insulin resistance at the age of some weeks. Then they develop hyperglycemia and reduced insulin secretion, which is typical for T2DM in humans [19]. Despite the short lifetime of db/db mice the model can be useful to study the pathogenesis of T2DM complications, in particular diabetic nephropathy, endothelial dysfunction and impaired wound healing [20-22].

Zucker fatty rats (ZFR) have a spontaneous mutation in leptin receptor gene, which leads to hyperphagia and to development of obesity, hyperinsulinemia and hypertension but not to diabetes [5, 23]. Zucker diabetic fatty (ZDF) rats, bred by inbreeding of ZFR, are less obese but demonstrate the larger insulin resistance and hyperglycemia [24]. Spontaneous T2DM develops in male ZDF rats leading to the complications typical for the late stages of diabetes in humans, which makes this model useful to study the pathogenesis of T2DM [18].

The example of animal models of T2DM without obesity is Goto-Kakizaki (GK) rats, which were bred by inbreeding of Wistar rats by hyperglycemia trait. GK rats are not obese but demonstrate impaired insulin secretion and insulin resistance [25]. This model can be used to study the efficiency of antidiabetic drugs [26], as well as for the study of the long-term complications of T2DM, such as retinopathy, nephropathy and peripheral neuropathy [18, 23]. The main limitation of the models of spontaneous diabetes is a high cost of housing and breeding of the rodents of the certain line.

Models of nutritionally induced DM. There are some models of experimental DM based on the change of nutritional status of animals to develop obesity and increased risk of T2DM. One of such models is a high-fat diet (HFD) which is used to induce obesity and insulin resistance in rodents [27]. This model is considered as imitating the Western diet, which contains more saturated acids that is recommended [28]. HFD

BIOLOGY AND BIOTECHNOLOGY

is often combined with administration of the low dose of STZ (25-35 mg/kg) that promotes faster and more stable induction of T2DM [29]. The HFD/STZ model is believed to imitate the pathogenesis of T2DM (from insulin resistance to β-cell dysfunction) as well as the complications of chronic DM such as cardiovascular, renal, and liver damage [30, 31].

Conclusions: The animal models of diabetes play an important role in modern biomedical research, making a background for the detailed study of pathogenesis, progression, and complications of both insulin-dependent and insulin-resistant diabetes. The variety models that differ in methods of diabetes induction and phenotype, indicate the complexity of pathobiochemical processes which make the basis for development of DM. A thorough understanding of advantages and limitations of each model is key for the informed choice of experimental design and interpretation of the data of experiment. It allows researchers to develop the effective therapeutic strategy aiming to prevent the development and progression of DM and its complications, as well as to evaluate the potential risks and benefits of the new therapeutic approaches before clinical trials.

References:

[1] Chatzigeorgiou A., Halapas A., Kalafatakis K., Kamper E. The use of animal models in the study of diabetes mellitus. In Vivo. 2009. 23(2). P.245-58.
[2] Brito-Casillas Y., Melián C., Wägner A.M. Study of the pathogenesis and treatment of diabetes mellitus through animal models. Endocrinol Nutr. 2016. 63(7). P.345-53.
[3] Rostène W., De Meyts P. Insulin: A 100-Year-Old Discovery with a Fascinating History. Endocr Rev. 2021. 42(5) P.503-527.
[4] Srinivasan K., Ramarao P. Animal models in type 2 diabetes research: an overview. Indian J Med Res. 2007. 125(3). P.451-472.
[5] Singh R., Gholipourmalekabadi M., Shafikhani S.H. Animal models for type 1 and type 2 diabetes: advantages and limitations. Front. Endocrinol. (Lausanne). 2024. 15. 1359685.
[6] Kurup S., Bhonde R.R. Combined effect of nicotinamide and streptozotocin on diabetic status in partially pancreatectomized adult BALB/c mice. Horm Metab Res. 2000. 32(8). P.330-334.
[7] Sakata N., Yoshimatsu G., Tsuchiya H., et al. Animal models of diabetes mellitus for islet transplantation. Exp Diabetes Res. 2012. 2012:256707.
[8] Yu Y.B., Bian J.M., Gu D.H. Transplantation of insulin-producing cells to treat diabetic rats after 90% pancreatectomy. World J Gastroenterol. 2015. 21(21). P.6582-6590.
[9] Ighodaro O.M, Adeosun A.M, Akinloye O.A. Alloxan-induced diabetes, a common model for evaluating the glycemic-control potential of therapeutic compounds and plants extracts in experimental studies.

BIOLOGY AND BIOTECHNOLOGY

Medicina (Kaunas). 2017. 53(6). P.365-374.
[10] King A.J. The use of animal models in diabetes research. Br J Pharmacol. 2012. 166(3). P.877-894.
[11] Ankur R., Ali Shahjad A. Alloxan Induced Diabetes: Mechanisms and Effects. Int J Res Pharm Biomed Sci. 2012. P.819-823.
[12] Lenzen S. The mechanisms of alloxan- and streptozotocin-induced diabetes. Diabetologia. 2008. 51(2). P.216-26.
[13] Rais N., Ved A., Ahmad R. et al. Model of Streptozotocin-nicotinamide Induced Type 2 Diabetes: a Comparative Review. Curr. Diabetes Rev. 2022. 18(8). 171121198001.
[14] Nahdi A.M.T.A., John A., Raza H. Elucidation of Molecular Mechanisms of Streptozotocin-Induced Oxidative Stress, Apoptosis, and Mitochondrial Dysfunction in Rin-5F Pancreatic β-Cells. Oxid Med Cell Longev. 2017. 2017. 7054272.
[15] Furman B.L. Streptozotocin-Induced Diabetic Models in Mice and Rats. Curr. Protoc. 2021. 1(4), P.78.
[16] Akinlade O.M., Owoyele B.V., Soladoye A.O. Streptozotocin-induced type 1 and 2 diabetes in rodents: a model for studying diabetic cardiac autonomic neuropathy. Afr. Health Sci. 2021. 21(2). P.719-727.
[17] Sevda Gheibi, Khosrow Kashfi, Asghar Ghasemi. A practical guide for induction of type-2 diabetes in rat: Incorporating a high-fat diet and streptozotocin Biomedicine & Pharmacotherapy. 2017. 95. P. 605-613.
[18] Martín-Carro B., Donate-Correa J., Fernández-Villabrille S. et al. Experimental Models to Study Diabetes Mellitus and Its Complications: Limitations and New Opportunities. Int J Mol Sci. 2023. 24(12). 10309.
[19] Wang Y.W., Sun G.D., Sun J. et al. Spontaneous type 2 diabetic rodent models. J Diabetes Res. 2013. 2013. 401723.
[20] Tesch G.H., Lim A.K. Recent insights into diabetic renal injury from the db/db mouse model of type 2 diabetic nephropathy. Am J Physiol Renal Physiol. 2011. 300(2). P.301-310.
[21] Beck L., Su J., Comerma-Steffensen S. et al. Endothelial Dysfunction and Passive Changes in the Aorta and Coronary Arteries of Diabetic db/db Mice. Front Physiol. 2020. 11. 667.
[22] Trousdale R.K., Jacobs S., Simhaee D.A. et al. Wound closure and metabolic parameter variability in a db/db mouse model for diabetic ulcers. J Surg Res. 2009. 151(1). P.100-107.
[23] Yagihashi S. Contribution of animal models to diabetes research: Its history, significance, and translation to humans. J Diabetes Investig. 2023. 14(9). P.1015-1037.
[24] Pang Y.L., Hu J.W., Liu G.L., Lu S.Y. Comparative medical characteristics of ZDF-T2DM rats during the course of development to late stage disease. Animal Model Exp Med. 2018. 1(3). P.203-211.
[25] Guest P.C. Characterization of the Goto-Kakizaki (GK) Rat Model of Type 2 Diabetes. Methods Mol Biol. 2019. 1916. P.203-211.
[26] Akash M.S., Rehman K., Chen S. Goto-Kakizaki rats: its suitability as non-obese diabetic animal model for spontaneous type 2 diabetes mellitus. Curr Diabetes Rev. 2013. 9(5). P.387-96.
[27] Lackey D.E., Lazaro R.G., Li P. et al. The role of dietary fat in obesity-induced insulin resistance. Am J Physiol Endocrinol Metab. 2016. 311(6). P.989-997.

BIOLOGY AND BIOTECHNOLOGY

[28] Stott N.L., Marino J.S. High Fat Rodent Models of Type 2 Diabetes: From Rodent to Human. Nutrients. 2020. 12(12). 3650.
[29] Wickramasinghe A.S.D, Attanayake A.P., Kalansuriya P. Biochemical characterization of high fat diet fed and low dose streptozotocin induced diabetic Wistar rat model. J Pharmacol Toxicol Methods. 2022. 113. 107144.
[30] Guo X.X., Wang Y., Wang K. et al. Stability of a type 2 diabetes rat model induced by high-fat diet feeding with low-dose streptozotocin injection. J Zhejiang Univ Sci B. 2018. 19(7). P.559-569.
[31] Sharma G., Ashhar M.U., Aeri V., Katare D.P. Development and characterization of late-stage diabetes mellitus and -associated vascular complications. Life Sci. 2019. 216. P.295-304.