Diabetic Rat Model

66

Miroslaw Lukaszuk

Abstract

In following chapter we present the basic information about the use of diabetic rats in experimental models. Historic attempts of diabetic model development are discussed. Various breeds of diabetic rats are presented and characterized. Finally we outline organ-specific complications observed in diabetic rats and present our experience in nerve regeneration in Zucker Diabetic Fatty (ZDF) rat model.

Keywords

Diabetes Mellitus • Diabetic Rat Model • Glycaemia In Rats • Complications of Diabetes • Nerve Regeneration In Diabetes • Diabetic Neuropathy Model

Abbreviations

BB	Bio breeding
CD	Cluster of differentiation
CVD	Cardiovascular disease
DM	Diabetes mellitus
DP	Diabetes prone
DR	Diabetes resistant
GLUT	Glucose transporter
PBG	Postprandial blood glucose
SSEP	Somatosensory evoked potentials
STZ	Streptozotocin
T1D	Type 1 diabetes
T2D	Type 2 diabetes
ZDF	Zucker diabetic fatty

M. Lukaszuk, MD
General and Endocrine Surgery and Gastroenterological Oncology Department, H. Swiecicki University Hospital, Poznan, Poland
e-mail: mlukaszuk@ump.edu.pl

Historic Development of Diabetic Animals for Experimental Studies

Early studies using animal model of diabetes mellitus (DM) are dated back to the first half of twentieth century. After insulin (initially called

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pancreatin) had been discovered in 1921 by Frederick Banting and Charles Best and its role in pathogenesis of hyperglycemia a year later, a new need for metabolic models arose in research. Until 1950s dogs, rabbits and rats after surgical procedure of pancreatectomy (pancreas removal) were used for experimental purposes. They expressed a total lack of internal insulin and served as models of what we call now type 1 diabetes [1]. This situation changed in 1950s, when Dr. Douglas Coleman from The Jackson Laboratory observed, that some of his extremely obese mice expressed insulin resistance and high blood glucose levels. Backcrossing of selected individuals resulted successfully in the first line of inbred species with type 2 diabetes phenotype [2]. In 1960s eye of science turned to detailed explanation of metabolic pathways in diabetes based on alloxan-induced rat model studies [3, 4]. Following years brought a variety of rodent experimental models of diabetes mellitus and its complications. Rats contributed greatly to understanding pathologic pathways and treatment of diabetes, as literally thousands of studies using diabetic strains have been conducted. It's noteworthy, no animal model fully mimicking pathology of human diabetes has been developed so far. An appropriate model should always be selected and used according to the individual study design.

Type 1 Diabetes Rat Models

Type 1 diabetes mellitus (T1D) in its natural history is characterized by a selective autoimmune destruction of β cells in pancreatic islets. This results in pancreatic insufficiency and a direct lack of endogenous insulin. In experimental animals this result can be achieved by either presence of insulitis-triggering genes conserved in strains through inbreeding (spontaneous diabetes) or administration of toxic agents in the genetic-competent breeds (induced diabetes). Pancreatectomy is nowadays the least usable method of diabetes induction, due to its technical complicity and possible unwanted complications of initial procedure.

Bio Breeding (BB) Rat

This model of spontaneous diabetes was developed in Bio Breeding Laboratories (Ottawa, Canada) in 1974 and is the most frequently studied T1D rat model. It comprises of two diabetes-prone (DP) colonies: inbred BBDP/Wor and outbred BBdp. Diabetes resistant BBDR rats are also available as controls. The usual age of diabetes onset is 8–14 weeks, sex-independent. Absolute insulinopenia, hyperglycaemia, polyuria, polydipsia and weight loss are present. Ketoacidosis is severe, and resembles status typically found in human type 1 disease. Exogenous insulin is necessary for survival. Autoimmune thyroiditis is commonly found, but rats do not become hypothyroidic. This model is compromised by immunopathologic CD4+ and CD8+ T lymphocyte deficits due to a severely reduced life span of peripheral T cells, not found in natural course of human T1D. Diabetes in BBDP rats is closely associated with depletion of rat-specific ART2+ T-reg cells and can be prevented by their transfusion [5–7].

Long Evans Tokushima Lean (LETL) and Komeda Diabetic Prone (KDP) Rats

LETL was the first rat model of spontaneous autoimmune T1D without coexisting lymphopenia (developed 1991). Incidence of diabetes in colonies was however low, 15–20 %. Following years of selected breeding gave origin to the KDP substrain exhibiting high incidence of 70 %. Insulitis is mild to severe with the onset usually at 17–32 weeks of age. Course of diabetic lesions is moderate. Lymphocytic inflammation is present in both thyroid gland and kidneys [7].

LEW.1AR1/Ztm-iddm Rat

The strain was selected in 2001 from a regular colony of Lewis rats at the Institute of Laboratory Animal Science of Hannover Medical School.

Spontaneous diabetes onset is observed usually at the age of 8–9 weeks, preceded by a week-long insulitis. Incidence is around 60 % and equal in both genders. The animals do not exhibit other autoimmune diseases or lymphopenia. Overt diabetes is moderate with good survival [6].

Chemically-Induced Diabetes in Rat

Several drugs have been tested and used for diabetes induction in experimental studies: alloxan, streptozotocin, vacor (rodenticide), dithizone, 8-hydroxyquinolone and others. From all the toxins streptozotocin (STZ) confirmed its position as a primary diabetes-inducing agent [5]. STZ (2-deoxy-2-(3-(methyl-3-nitrosoureido)-D-GLUCOPYRANOSE) is a nitrourea derivative obtained from *Streptomyces achromogenes*. In vivo it expresses a strong alkylating ability and is a source of free radicals. The particles are similar to glucose and are heavily taken up by GLUT2 transporter in pancreatic cells. Fasting animals are more susceptible as STZ competes with glucose. When administered intravenously or intraperitoneally a single dose 50–65 mg/kg STZ produces diabetes in selected rat strains (commonly Wistar rats) through direct toxicity to β cells. Hyperglycaemia becomes stable after 5 days. Alternatively multiple lower doses 20–40 mg/kg per day over consecutive 5 days can be administered to induce insulitis and a subsequent β cell destruction to achieve similar pathophysiological effect. In some cases spontaneous regeneration of pancreatic cells occurs [5, 6, 8].

Alloxan (2,4,5,6-tetraoxypyrimidine;5,6-dioxyuracil) has diabetes-inducing capability mainly attributed to the formation of free radicals in pancreatic islets and oxidation of essential –SH groups in glucokinase. Usual single dose ranges 40–200 mg/kg with intraperitoneal and subcutaneous administration up to three times higher than intravenous. Alloxan has a very restricted diabetogenic dose and even light overdosing is considered extremely toxic to liver and kidneys. Therefore alloxan has been widely replaced by STZ in experimental models [6, 8].

Virally-Induced Diabetes in Rat

Several viruses have been tested as diabetes inducers in rodents. Coincidence between diabetes and virus infection is known since late 1960s. Coxsackie B virus, encephalomyocarditis virus or Kilham rat virus infection triggers insulitis and β cells destruction either due to direct effect of viral clones or an autoimmune response targeting pancreatic cells. Model is fluctuant, however it can be used to determine potential role of those viruses in type 1 diabetes pathology [6].

Type 2 Diabetes Rat Models

Type 2 diabetes mellitus (T2D, 90 % of all cases of DM) is a complex metabolic disease, mainly characterized by insulin resistance and insufficient secretion of endogenous insulin (relative lack, β cells failure). Obesity is present in almost all cases. Skeletal muscle defect is a primary cause for clinical resistance to insulin in human. Compensatory hyperinsulinemia is switched on to maintain glucose tolerance. After this mechanism becomes ineffective, hyperglycaemia occurs, but it is usually milder than in type 1 disease. T2D is frequently accompanied by a variety of concomitant disorders, like dyslipidaemia, hypertension, atherosclerosis and cardiovascular disease (CVD). This represents a typical phenotype of polimetabolic syndrome (syndrome X) [9]. Majority of rodent models of T2D are obese to reflect condition in human.

Zucker Diabetic Fatty (ZDF) Rat

The colony was selected from obese Zucker strain. ZDF is a monogenic inbred model with fa/fa genotype representing dysfunction of hypothalamic leptin receptor (leptin resistance). It's manifested by insatiable hunger and habitual stationary life style, that lead to fast weight gain, obesity and insulin resistance. Increased mass of fat tissue and inherited β cells expansion failure express as relative lack of endogenous insulin.

Defects in pancreatic GLUT2 and muscular GLUT4 glucose transporters appear to play additional role in diabetes onset. Animals become obese at 4 weeks of age. Impaired glucose tolerance, hyperinsulinaemia, hyperlipidaemia and hypertension are exhibited usually 4–6 weeks later in males. Females do not develop full image of diabetes. Histologically ZDF rats do not display the same pathology (amyloid deposits) in pancreatic islets as it is observed in human T2D [6, 9].

Goto-Katazaki (GK) Rat

GK rat model was developed in 1976 by selective breeding of Wistar rats with highest blood glucose levels. Diabetes is moderate, hyperglycaemia is observed in adult life. Insulin resistance in both skeletal muscles and liver as well as impaired insulin secretion in pancreas are present at the onset. Rats do not exhibit hyperlipidaemia and obesity [5, 10].

Otsuka Long-Evans Tokushima Fatty (OLETF) Rat

OLETF rat was developed in 1984 from Long-Evans colony exhibiting glucose intolerance. The model is polygenic. Obesity is mild, with stronger expression in males. Hyperglycaemia has late onset at the age of 18 weeks. The wild-type controls are not available for this model [5, 6].

Muridae Family

Due to taxonomic confusions some authors include Shafrir Israeli sand rat (properly desert gerbil, *Psammomys obesus*) and Nile grass rat (*Arvicanthis niloticus*) into the pool of diabetic rat models. In fact, these particular species of *Muridae* family don't belong to the genus *Rattus*, but they are valuable rodent models of specific diabetic phenotype. In natural conditions gerbils have vegetarian diet. When fed with fat-rich chow they develop obesity, hyperglycaemia and insulin resistance followed by hyperlipidaemia, atherosclerosis and overt type 2 diabetes. The strain is useful for studying diet impact in diabetes [5]. *Arvicanthis* is a novel complex model of metabolic syndrome. Obesity is spontaneous, along with dyslipidaemia and hyperglycaemia on a normal laboratory chow diet. These are followed by β cells failure, atherosclerosis, liver steatosis, abdominal fat accumulation, nephropathy and hypertension. The overt diabetes is observed by 1 year of age [11].

Other-Type Diabetic Rat Models

Transgenic and Knock-Out Metabolic Models

This relatively new technique is used to create models with single genetic impairment. Virus-assisted gene transfer allows to change enzyme or other protein composition and effect or even knock-out some metabolic pathways. Modified genes are incorporated into the zygote and born pups exhibit desired phenotype according to the study design. Models with insulin under- and overexpression are possible to develop. Studies using modified insulin receptor, insulin receptor substrate (IRS), glucokinase, insulin-like growth factor 1 (IGF1) and glucose transporter 4 (GLUT4) were published [5]. Some knock-out models require high fat feeding to exhibit overt diabetes [6].

Gestational Diabetes Models

Streptozotocin-induced gestational diabetes in outbred non-diabetic rats (i.e., Wistar) is a useful and popular model for studies. An usual single dose of 40–50 mg/kg is given intravenously within the first week of gestation. Clinical diabetes can be also triggered prior to pregnancy. In a study comparing dams with clinical diabetes during pregnancy a group with blood glucose levels >300 mg/kg exhibited significantly higher rate of pre- and postimplantation loss than a group with glycaemia 120–300 mg/dl. Mild diabetes in the latter group was induced by a single intraperitoneal dose of STZ (70 mg/kg) on day 5 of dam's life. Both

groups presented intrauterine growth restriction of the offsprings, which is opposite to human, probably due to the short time of pregnancy in rats [12]. According to other studies streptozotocin administered in pregnant rat females caused also impaired glucose tolerance in fetuses and decreased insulin secretion in later life, however a considerable variability in glucose concentrations was observed [13, 14]. An interesting study showed, that Wistar rat embryos (low risk of diabetes) when transferred to the uterus of Goko-Katazaki rat exhibited hyperglycaemia in adult life. Thus the intrauterine environment appears to play a crucial role in development of diabetes in later life [15].

Blood Glucose Levels in Diabetic Rats

Glycaemia in diabetic rats is a strong variable, that requires careful monitoring. In non-diabetic rats a mean postprandial blood glucose (PBG) concentration was reported to oscillate around 102 mg/dl [16]. Fasting and PBG levels in non-diabetic Wistar and diabetic ZDF rats noted in our laboratory are presented in Figs. 66.1 and 66.2. We observed a daily amplitude in diabetic rat blood glucose concentration, with its values significantly higher in the afternoon than in the morning. Similar tendency but not significant was observed in non-diabetic rats.

Blood Acquisition Method

A drop of blood for analysis can be obtained from tail capillary vessels. In this technique a person restrains rat with one hand using a plastic cone, with a tip of the cone cut off to allow the animal access to the fresh air. Tail needs to be warmed up by immersing it in warm water for few seconds. After tail is dried with a tissue, a small incision or puncture is made at the tail tip using a sterile lancet or a needle. "Milk-out"

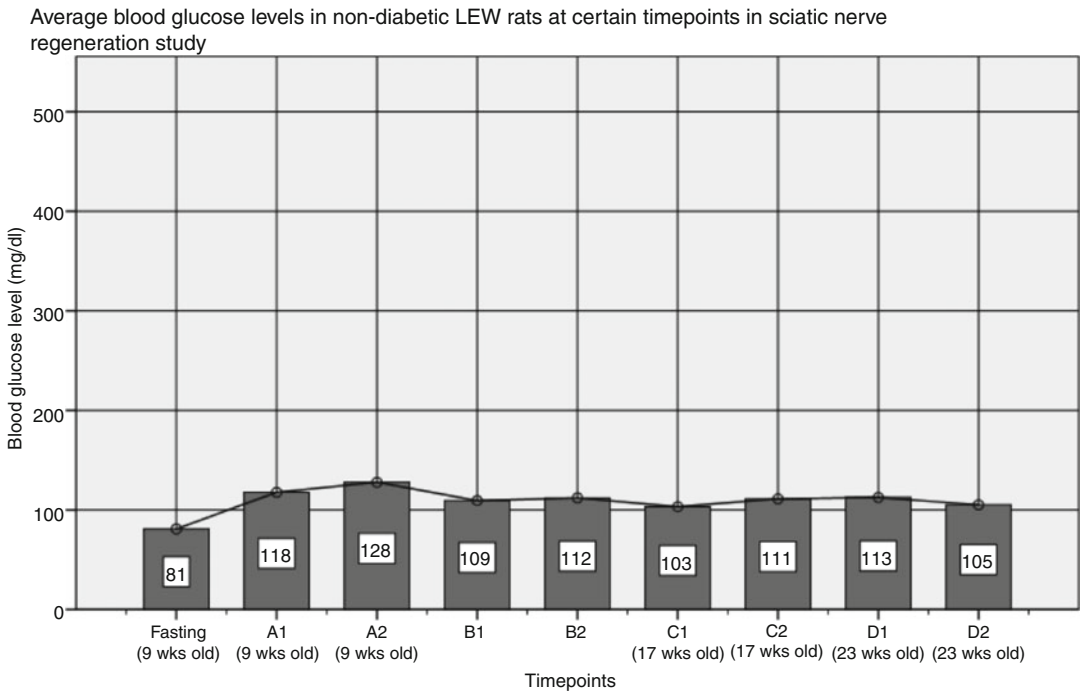

Fig. 66.1 Blood glucose concentration in non-diabetic Lewis rats observed in sciatic nerve repair study, average age at the procedure – 10 weeks and 4 days: fasting – after 16 h of fasting, 9 weeks old; (*A*) before the procedure, around 9 weeks old; (*B*) within 3 days after the procedure, (*C*) at 6 weeks after the procedure (around 17 weeks old), (*D*) at 12 weeks after the procedure (around 23 weeks old); (*1*) indicates blood acquisition in the morning (8–9 A.M.), (*2*) indicates blood acquisition in the afternoon (4–5 P.M.)

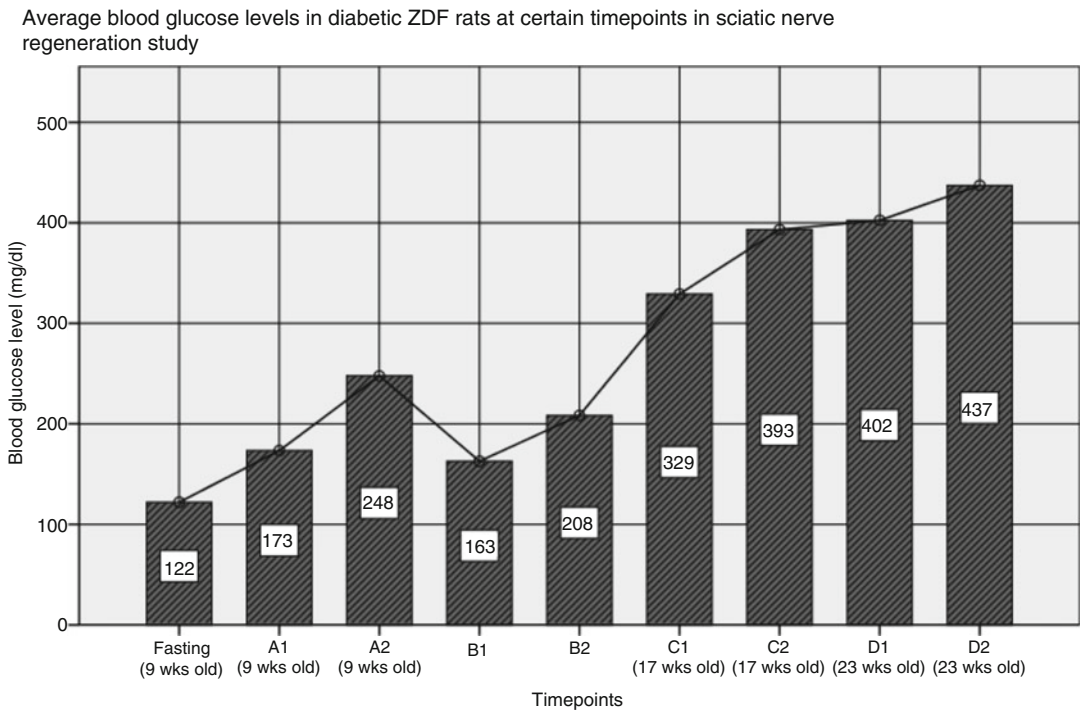

Fig. 66.2 Blood glucose concentration in diabetic ZDF rats observed in sciatic nerve repair study, average age at the procedure – 10 weeks and 5 days: fasting – after 16 h of fasting, 9 weeks old; (*A*) before the procedure, around 9 weeks old; (*B*) within 3 days after the procedure, (*C*) at 6 weeks after the procedure (around 17 weeks old), (*D*) at 12 weeks after the procedure (around 23 weeks old); (*1*) indicates blood acquisition in the morning (8–9 A.M.), (*2*) indicates blood acquisition in the afternoon (4–5 P.M.)

technique helps to obtain full droplet, that should be directly transferred onto a glucometer strip. Blood acquisition can be repeated daily from the same tail tip.

Organ-Specific Complications of Diabetes in Rats

Chronic hyperglycaemia or hyperinsulinaemia lead to a variety of complications. Some of the diabetic rat strains described above represent valuable models of complications of diabetes. Due to the slow development of disease Goto-Kakizaki rat was used in studies of ongoing diabetic complications: nephropathy [17], peripheral neuropathy [18] and retinal lesion [19]. Diabetic neuropathy in T1D was studied using Bio breeding BB/Wor rats [20]. Studies on chronic renal disease in T2D were conducted with use of OLETF rat model, allowing 50 weeks-long observation [21]. According to Cefalu and coauthors microvascular and cardiovascular complications are already present during the pre-diabetic metabolic syndrome initial phase, before the clinical onset of diabetes (i.e., in human increased risk for CVD 15 years before the diagnosis of DM) [9]. No animal model of diabetic complications fully reflects their nature seen in human. Therefore model selection should be always adjusted to the study design. Ideally experiments should be performed using various complementary animal models of diabetic complications to achieve rewarding results.

Nerve Regeneration in Diabetes-Induced Neuropathy

Peripheral neuropathy is one of the most commonly found complication of diabetes. Several rodent and non-rodent animal models were proposed to be used to study this topic. Pathogenesis of this particular complication is yet not fully understood, therefore no single experimental model of neuropathy reflecting human condition is known. Generally degeneration of all fiber types in affected nerve occurs in conjunction with chronic hyperglycaemia and hyperinsulinaemia. Clinical sensory lost, increased vibration and thermal perception thresholds, paresthesia, hyperalgesia and spontaneous pain are present as a result [22]. These symptoms are very subjective and their assessment is difficult in experimental studies using animals. Thus design of such study should be aimed at evaluation of objective results, like histopathology, direct observation of nerve lesion or regeneration, neuronal electrophysiology measurements or animal-independent clinical tests based on reflexes. Non-diabetic controls are needed for comparison.

In our study we used ZDF rats to evaluate sciatic nerve regeneration after injury (20 mm gap) and subsequent repair by either autologous nerve graft or a novel technique using autologous hollow epineural sheath conduit graft (experimental groups) (Fig. 66.3). A respective control groups of non-diabetic Lewis rats were treated according to the same protocol. Number of animals in every group was eight. Criteria of inclusion for ZDF rats were: glycaemia >200 mg/dl (twice) and glycemia after 16 h of fasting >110 mg/dl (overt diabetes). Animals were not treated with oral antidiabetics or exogenous insulin. Lewis rats were matched with ZDF rats for similar weight at the day of surgical procedure. Nerve regeneration was assessed 12 weeks after the operation. Relative results showed impaired regeneration in diabetic rats, when compared to the control groups. Clinical sensory and motor tests gave 22–29 % worse results in ZDF groups. Conduction velocity in regenerated nerve in diabetic animals was reduced by 4–9 % at week 12 after the procedure (assessed in somatosensory evoked potentials (SSEP) protocols) (unpublished data). Denervation atrophy in surgically reinervated muscles was significantly milder in non-diabetic rats (micromorphometric muscle fiber assessment), ending up with 53 % of control fiber cross-sectional area retained in Lewis vs 42 % in ZDF rats [23]. To the best of our knowledge this is the first study using experimental animal type 2 diabetes model in assessment of peripheral nerve regeneration. It is also the first application of epineural sheath conduit in nerve repair under diabetic conditions.

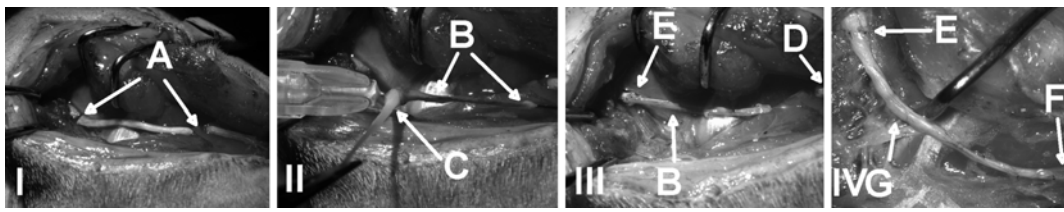

Fig. 66.3 Surgical procedure of sciatic nerve gap creation and repair in ZDF diabetic rat: I – 20 mm gap is created in right sciatic nerve, II – epineural sheath conduit is created from the dissected nerve segment, III – epineural sheath conduit during implantation, proximal coaptation is performed, IV – nerve regeneration at 12 weeks after the procedure, ingrowth of nerve fibers can be observed in the conduit; (*A*) incisions made in sciatic nerve, (*B*) epineural sheath conduit, (*C*) nerve fibers, (*D*) distal nerve stump, (*E*) proximal coaptation, (*F*) distal coaptation, (*G*) nerve fascicle inside the conduit

References

1. Bliss M. The discovery of insulin. Chicago: University of Chicago Press; 2007.
2. Coleman DL. A historical perspective on leptin. Nat Med. 2010;16(10):1097–9. doi:10.1038/nm1010-1097.
3. Russfield AB. Experimental endocrinopathies. Methods Achiev Exp Pathol. 1975;7:132–48.
4. Fischer LJ, Rickert DE. Pancreatic islet-cell toxicity. CRC Crit Rev Toxicol. 1975;3(2):231–63.
5. Rees DA, Alcolado JC. Animal models of diabetes mellitus. Diabet Med. 2005;22(4):359–70.
6. King AJ. The use of animal models in diabetes research. Br J Pharmacol. 2012;166(3):877–94. doi:10.1111/j.1476-5381.2012.01911.x.
7. Mordes JP, Bortell R, Blankenhorn EP, Rossini AA, Greiner DL. Rat models of type 1 diabetes: genetics, environment, and autoimmunity. ILAR J. 2004;45(3):278–91.
8. Szkudelski T. The mechanism of alloxan and streptozotocin action in B cells of the rat pancreas. Physiol Res. 2001;50(6):537–46.
9. Cefalu WT. Animal models of type 2 diabetes: clinical presentation and pathophysiological relevance to the human condition. ILAR J. 2006;47(3):186–98.
10. Janssen U, Vassiliadou A, Riley SG, Phillips AO, Floege J. The quest for a model of type II diabetes with nephropathy: the Goto Kakizaki rat. J Nephrol. 2004;17(6):769–73.
11. Noda K, Melhorn MI, Zandi S, Frimmel S, Tayyari F, Hisatomi T. An animal model of spontaneous metabolic syndrome: Nile grass rat. FASEB J. 2010;24(7):2443–53. doi:10.1096/fj.09-152678. Epub 2010 Mar 24.
12. Kiss AC, Lima PH, Sinzato YK, Takaku M, Takeno MA, Rudge MV, et al. Animal models for clinical and gestational diabetes: maternal and fetal outcomes. Diabetol Metab Syndr. 2009;1(1):21. doi:10.1186/1758-5996-1-21.
13. Caluwaerts S, Holemans K, van Bree R, Verhaeghe J, Van Assche FA. Is low-dose streptozotocin in rats an adequate model for gestational diabetes mellitus? J Soc Gynecol Investig. 2003;10(4):216–21.
14. Van Assche FA, Aerts L, Holemans K. The effects of maternal diabetes on the offspring. Baillieres Clin Obstet Gynaecol. 1991;5(2):485–92.
15. Gill-Randall R, Adams D, Ollerton RL, Lewis M, Alcolado JC. Type 2 diabetes mellitus–genes or intrauterine environment? An embryo transfer paradigm in rats. Diabetologia. 2004;47(8):1354–9. Epub 2004 Jul 17.
16. Wang Z, Yang Y, Xiang X, Zhu Y, Men J, He M. Estimation of the normal range of blood glucose in rats. Wei Sheng Yan Jiu. 2010;39(2):133–7. 142.
17. Janssen U, Phillips AO, Floege J. Rodent models of nephropathy associated with type II diabetes. J Nephrol. 1999;12(3):159–72.
18. Murakawa Y, Zhang W, Pierson CR, Brismar T, Ostenson CG, Efendic S, Sima AA. Impaired glucose tolerance and insulinopenia in the GK-rat causes peripheral neuropathy. Diabetes Metab Res Rev. 2002;18(6):473–83.
19. Sone H, Kawakami Y, Okuda Y, Sekine Y, Honmura S, Matsuo K, et al. Ocular vascular endothelial growth factor levels in diabetic rats are elevated before observable retinal proliferative changes. Diabetologia. 1997;40(6):726–30.
20. Zhang W, Kamiya H, Ekberg K, Wahren J, Sima AA. C-peptide improves neuropathy in type 1 diabetic BB/Wor-rats. Diabetes Metab Res Rev. 2007;23(1):63–70.
21. Lee MY, Shim MS, Kim BH, Hong SW, Choi R, Lee EY, et al. Effects of spironolactone and losartan on diabetic nephropathy in a type 2 diabetic rat model. Diabetes Metab J. 2011;35(2):130–7. doi:10.4093/dmj.2011.35.2.130. Epub 2011 Apr 30.
22. Islam MS. Animal models of diabetic neuropathy: progress since 1960s. J Diabetes Res. 2013;2013:149452. doi:10.1155/2013/149452. Epub 2013 Jul 29.
23. Lukaszuk M, Kwiecień G, Madajka M, Uygur S, Drews M, Siemionow M. Repair of the peripheral nerve gap with epineural sheath conduit to prevent muscle denervation atrophy in the diabetic rat model. Pol Przegl Chir. 2013;85(7):387–94. doi:10.2478/pjs-2013-0059.