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Chemically Induced of Diabetes Mellitus in Experimental Rats: Mechanisms, Protocols, and Translational Challenges

Rupesh Soni[1]*, Rahul Trivedi[2], Krishnapal Nagda[3]

[1] Professor and Principal, B R Nahata College of Pharmacy, Mandsaur University, Mandsaur, Madhya Pradesh, India.
[2] Professor, Department of Pharmacy, Sumandeep Vidyapeeth, Vadodara, Gujrat, India.
[3] Assistant Professor, B R Nahata College of Pharmacy, Mandsaur University, Mandsaur, Madhya Pradesh, India.
*Corresponding author: Rupesh Soni, Professor and Principal, B R Nahata College of Pharmacy, Mandsaur University, Mandsaur, Madhya Pradesh, India.

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ABSTRACT

Background: Diabetes Mellitus (DM) continues to be a major issue globally and hence rigorous preclinical testing and experimenting with new drugs is of paramount importance. Laboratory animals help to a large extent in this endeavor as their use in experiments is justified through their cost-effectiveness and reproducibility.

Objective: The objective of the current review is to provide an overview and mechanistic assessment of major agent causing diabetes in laboratory rats suggested to be used in various studies.

Main Mechanisms: The major agents causing diabetes include streptozotocin (STZ) and alloxan which work through the stimulation of transmembrane glucose transporter of GLUT-2. The main mechanism of STZ is its entry into pancreatic ducts where it triggers DNA alkylation along with hyperactivation of PARP and exhaustion of NAD. Alloxan on the other hand initiates rapid redox cycling inside the cells generating high concentrations of ROS. Other agents include dithizone, which inactivates insulin due to its ability to chelate zinc ions besides gold thioglucose and monosodium glutamate which act on the hypothalamus causing the diabetic syndrome.

Practical implications: High-dose approaches can successfully bring to life the total lack of insulin seen in Type 1 diabetes (T1DM), whereas low-dose settings together with nutrition changes replicate the insulin resistance and the gradual destruction of β-cells typical for Type 2 diabetes (T2DM) [9]. The task of optimizing these models includes overcoming the challenges of strain variations, different mortality rates, and the issue of spontaneous β-cell regeneration.

Keywords: None.

Introduction

Diabetes mellitus refers to a diverse group of metabolic disturbances distinguished by chronic high blood sugar levels due to either a defect in one or both of the processes of insulin production and insulin utilization [11]. The incidence of both type 1 and 2 diabetes is increasing globally, causing enormous strain on healthcare systems and stimulating demand for more innovative treatment, technology, and preventive measures [12]. While in vitro techniques can provide limited mechanistic information, understanding diabetes requires whole-animal in vivo studies due to the complexity of diabetes that manifests itself through organ interactions, micro- and macroscopic metabolic shifts, and other systemic regulators [13].

In preclinical studies, Wistar and Sprague-Dawley rats serve as preeminent mammals for metabolism analysis [14]. The characteristics of these strains help provide predictable metabolic parameters that are similar to human reactions when exposed to certain diets. Most importantly, these strains are perfect for studying the development of diabetes complications such as diabetic nephropathy (which involves progressive formation of albumin in the urine and glomerulosclerosis) [15], diabetic neuropathy (characterized by changes in nerve conduction speed and thermal hyperalgesia) [16], and diabetic retinopathy (where the changes in micro-vessels and retinal cells occur) [17].

To create diabetic traits in these rodent species, researchers opt for surgical versus genetic or chemical induction methods. Surgical methods (such as partial or complete pancreatectomy) pose technical challenges, require low-technology equipment, and present additional complications of exocrine pancreatic insufficiencies [18]. Genetic models (for example, the Zucker Diabetic Fatty rat) have the advantage of uniformity of disease progression, but incur significant cost, and are difficult to breed, and the strains may have genotypes that do not represent the complex, multifactorial causes of human diabetes [19]. Thus, chemical induction methods are the method of choice for preclinical testing [20]. Their most important advantages are the low cost, the ease of application, and the ability to control the phenotypic outcome through proper dose and diet adjustment.

Core Chemical Induction & Mechanistic Underpinnings

1. Streptozotocin (STZ)

Chemical structure and properties: Streptozotocin is a natural glucosamine-nitrosourea compound extracted from Streptomyces achromogenes [21]. Structurally speaking, there is a very toxic nitrosourea component linked to a glucose molecule (2-deoxy-2-(3-methyl-3-nitrosoureido)-D-glucopyranose). Thanks to this particular structure, STZ is able to work as a D-glucose mimetic.

Mechanism of cellular entry: STZ has a selective pancreatic toxicity due to its particular capacity to utilize specific transport systems of pancreatic β-cells. As it is a glucose analogue, STZ is recognized by and binds selectively to the glucose transporter protein GLUT-2, which is a high-capacity and low-affinity transmembrane transporter [22]. All the other glucose transporters show weak affinity to this substance; however, GLUT-2 is extensively represented on cell membranes of rodent islet cells and less on hepatocytes and tubular cells [23]. Thus, STZ is quickly penetrated into the cytoplasm of the β-cell while neighboring α-cells stay unharmed.

Intracellular Cascades and Cytotoxicity: As soon as STZ enters the cells, it rapidly decomposes, leading to the commencement of a series of cytotoxic processes. The nitroso-urea part of STZ produces cation radicals and alkylates the genomic DNA at O6 and N7 positions of guanine bases [24]. The occurrence of DNA double-strand breaks leads to an activation of the nuclear repair enzyme poly(ADP-ribose) polymerase-1 (PARP-1) [25]. The significant PARP-1 activation uses up the cellular NAD+ since the involved processes require ATP. At the same time, STZ acts as a donor of NO, which bonds to the superoxide radical and generates peroxynitrite causing oxidative damage [26]. The pancreatic β-cells are sensitive to the oxidative damage because they have an extremely low level of antioxidant enzymes in the cells.

Induction Techniques Using Rats: A single large dose of 35-65 mg/kg of STZ, which can be administered by intravenous or intraperitoneal injection, causes complete insulin shortage in adult rats like it happens in type 1 diabetes. Before the use of STZ, it must be dissolved in ice-cold 0.1 M citrate buffer that has a specific pH of 4.5. In order to induce T2DM in rats, researchers have developed a protocol aiming to create damage in the peripheral tissue coupled with stratified dietary priming with a high-fat diet (containing 45-60% fat) for several weeks and followed by the administration of small sub-diabetogenic doses of STZ by intraperitoneal injection.

2. Alloxan

Chemical Structure and Properties: Alloxan (2,4,5,6-tetraoxypyrimidine) is a type of oxygenated pyrimidine that has a similar structure with both uric acid and glucose and is known to be reactive and unstable in an aqueous environment.

Mechanism of Cellular Entry: Like STZ, alloxan enters cells through GLUT-2 due to its structural similarities with the hydrophilic D-glucose ring. This compound does not seem to accummulate in tissues that do not have a high GLUT-2 density.

Intracellular Cascades and Cytotoxicity: The cytotoxic characteristics of alloxan are due to a series of reduction and oxidation processes. The reduction of alloxan to dialuric acid takes place in the presence of intracellular reducing factors, which are represented mainly by the cytosolic glutathione (GSH). Alloxan is afterward auto-oxidized into the initial compound. Alloxan also interacts directly with the -SH groups, thereby blocking the functioning of glucokinase (the main glucose receptor in beta-cells) and thus leading to cell death in the area.

Glucose Profile: The Triphasic Response: Systemic alloxan administration triggers a dynamic triphasic blood glucose curve in rats through 24–48 hours post-alloxan use: Phase 1 involves initial hyperglycemia (0–4 hours) due to a spike in hepatic glycogenolysis, Phase 2 has transitory hypoglycemia (6–12 hours) as a result of the death of pancreatic β-cells and flooding the blood with stored insulin, and Phase 3 is noted for permanent hyperglycemia (24–48 hours and beyond) indicating total destruction of the functional β-cell mass [23]. To avoid death during Phase 2 process, provision must be made to ensure that animals receive water supplemented with 10–20% sucrose for the first 24 hours post alloxan.

Administration protocols for the rats: Alloxan is administered to fasting rats within the dosage range of 40-150 mg/kg (40-60 mg/kg for i.v., 100-150 mg/kg for i.p./s.c.) [34]. Since alloxan has a narrow therapeutic window, it is imperative to ensure stricter than 12-24 hour fasting period to make sure intracellular glucose level has been brought down and there is minimum chance for competitive inhibition.

3. Minor & Emerging Diabetogenic Chemicals

Dithizone is a member of the thiocarbonyl family of semi-organic compounds and is a chelator. When injected intravenously at doses of 40-100 mg/kg, it binds with zinc ions (Zn2+) that are required for maintaining the stability and storage of hexamers of insulin in the intracellular granules, resulting in disintegration of its structure and acute distress.[25]

GTG, which belongs to the class of organogold compounds, is used to create neurogenic models of T2DM. If administered via the intraperitoneal route (dosing: 200-400 mg/kg), GTG penetrates the blood-brain barrier and accumulates in glucose-sensing cells of the VMH, giving rise to local necrotic lesions that disrupt appetite control, thus causing hyperphagia, obesity, and secondary insulin resistance.[26]

MSG is injected by subcutaneous route to young rats (at a dose of 2-4 mg/kg daily) in the first 10 days of life, so that it acts like a neuroexcitotoxin and selectively destroys sensitive neurons in the arcuate nucleus of the hypothalamus. This leads to leptin resistance, metabolic changes – obesity, and impaired glucose tolerance later in life.[27]

4. Comparative Analysis

Chemical Agent	Primary Mechanism of Action	Typical Rat Dosage	Targeted Type	Major Advantages / Limitations
Streptozotocin (STZ)	Selective GLUT-2 entry; DNA alkylation; PARP-1 hyperactivation; NAD+/ATP depletion.	T1DM: 35-65 mg/kg i.v./i.p. T2DM: 25-35 mg/kg i.p. + HFD	T1DM or T2DM	Adv: High reproducibility, stable hyperglycemia. Lim: Relatively expensive.
Alloxan	Selective GLUT-2 entry; redox cycling; massive ROS generation; glucokinase inhibition.	40-60 mg/kg i.v. 100-150 mg/kg i.p./s.c.	T1DM	Adv: Highly cost-effective; rapid onset. Lim: High mortality; narrow index; hypoglycemic risk.
Dithizone	Chelation of intracellular zinc (Zn2+) ions within secretory insulin granules.	40-100 mg/kg i.v.	Acute T1DM-like	Adv: Targeted granular disruption. Lim: Hyperglycemia often unstable/reversible.
Gold Thioglucose	Accumulates in VMH glucose-sensing cells; induces focal necrosis destroying satiety center.	200-400 mg/kg i.p.	T2DM / Obesity	Adv: Captures neurogenic obesity without genetic mutants. Lim: Severe brain lesions; variable glucose profiles.
Monosodium Glutamate	Neonatal excitotoxicity targeting the arcuate nucleus of the hypothalamus.	2-4 mg/g s.c. daily (neonatal days 1-10)	T2DM / MetS	Adv: Replicates intact but highly insulin-resistant islets. Lim: Long incubation; causes stunted growth.

Conclusion & Future Directions:

Rat models resulting from chemical reactions are some of the most important existing tools in modern studies of diabetes before clinical trials can commence, as they embody a cost-efficient approach while guaranteeing reproducible results. For example, STZ or alloxan act as landmarks in inducing acute toxicity via the glucose transport through GLUT-2. Apart from them, there are also some specialized substances like gold thioglucose and monosodium glutamate responsible for the induction of neurogenic obesity and metabolic syndrome. Future studies should concentrate on regulated standards in reporting with regard to parameters such as the pH of the vehicle used, and have strict rules regarding fasting and detailed reports about the diet of the animals involved in the study.

References

1. Schnedl WJ, Ferber S, Johnson JH, Newgard CB. STZ transport and cytotoxicity: specific enhancement in GLUT2-expressing cells. Diabetes. 1994;43(11):1326-1333.
2. Elsner M, Guldbakke B, Tiedge M, Munday R, Lenzen S. Relative importance of transport and alkylation for pancreatic beta-cell toxicity of streptozotocin. Diabetologia. 2000;43(12):1528-1533.
3. Pieper AA, et al. Nature Medicine. 1999;5(3):314-320.
4. Munday R. Biochemical Pharmacology. 1988;37(21):4095-4102.
5. Wolff SP, et al. Biochemistry. 1986;25(16):4541-4546.
6. Okamoto H. Molecular and Cellular Biochemistry. 1985;67(2):155-181.
7. West E, et al. Journal of Toxicology and Environmental Health. 1996;48(3):283-294.
8. Like AA, Rossini AA. Streptozotocin-induced pancreatic insulitis: new model of diabetes mellitus. Science. 1976;193(4251):415-417.
9. Reed MJ, et al. Metabolism. 2000;49(11):1390-1394.
10. Srinivasan K, Viswanad B, Asrat L, Kaul CL, Ramarao P. Combination of high-fat diet-fed and low-dose streptozotocin-treated rat: a model for type 2 diabetes and pharmacological screening. Pharmacological Research. 2005;52(4):313-320.
11. American Diabetes Association. Diagnosis and classification of diabetes mellitus. Diabetes Care. 2014;37(Suppl 1).
12. Wild S, Roglic G, Green A, Sicree R, King H. Global prevalence of diabetes: estimates for the year 2000 and projections for 2030. Diabetes Care. 2004;27(5):1047-1053.
13. Rees DA, Alcolado JC. Animal models of diabetes mellitus. Diabetic Medicine. 2005;22(4):359-370.
14. King AJF. The use of animal models in diabetes research. British Journal of Pharmacology. 2012;166(3):877-894.
15. Tesch GH, Allen TJ. Rodent models of streptozotocin-induced diabetic nephropathy. Nephrology. 2007;12(3):261-266.
16. Biessels GJ, et al. Diabetes/Metabolism Research and Reviews. 2014;30(5):343-356.
17. Lai AKW, Lo ACY. Journal of Diabetes Research. 2013;2013:106207.
18. Bone AJ, et al. Journal of Endocrinology. 1989;120(1):123-129.
19. Kurtz TW, et al. Hypertension. 1989;13(6 Pt 2):896-901.
20. Lenzen S. The mechanisms of alloxan- and streptozotocin-induced diabetes. Diabetologia. 2008;51(2):216-226.
21. Lenzen S, Munday R. Diabetologia. 1991;34(5):373-376.
22. Katsumata K, et al. Hormone and Metabolic Research. 1992;24(11):508-510.
23. Kadota I. Journal of Laboratory and Clinical Medicine. 1950;35(4):568-577.
24. Brecher G, Waxler SH. Proceedings of the Society for Experimental Biology and Medicine. 1949;70(3):498-501.
25. Olney JW. Brain lesions, obesity, and other disturbances in mice treated with monosodium glutamate. Science. 1969;164(3880):719-721.
26. Rossini AA, et al. Science. 1977;195(4281):880-882.
27. Whiteman M, et al. British Journal of Medicine. 2010;14(2):112-124.
28. Goren HJ, et al. Endocrinology. 2004;145(5):2145-2152.