Chemico-Biological Interactions 244 (2016) 49–63

Contents lists available at ScienceDirect

Chemico-Biological Interactions

journal homepage: www.elsevier.com/locate/chembioint

Challenges and issues with streptozotocin-induced diabetes – A clinically relevant animal model to understand the diabetes pathogenesis and evaluate therapeutics

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ARTICLE INFO

Article history:
Received 30 April 2015
Received in revised form
18 November 2015
Accepted 26 November 2015
Available online 2 December 2015

Keywords:
STZ
Streptozotocin
Diabetes

ABSTRACT

Streptozotocin (STZ) has been extensively used over the last three decades to induce diabetes in various animal species and to help screen for hypoglycemic drugs. STZ induces clinical features in animals that resemble those associated with diabetes in humans. For this reason STZ treated animals have been used to study diabetogenic mechanisms and for preclinical evaluation of novel antidiabetic therapies. However, the physiochemical characteristics and associated toxicities of STZ are still major obstacles for researchers using STZ treated animals to investigate diabetes. Another major challenges in STZ-induced diabetes are sustaining uniformity, suitability, reproducibility and induction of diabetes with minimal animal lethality. Lack of appropriate use of STZ was found to be associated with increased mortality and animal suffering. During STZ use in animals, attention should be paid to several factors such as method of preparation of STZ, stability, suitable dose, route of administration, diet regimen, animal species with

Abbreviations: Akt, serine/threonine kinase; ATP, adenosine triphosphate; cGMP, cyclic guanosine monophosphate; $CH3^{+}$, carbonium ion; CNS, central nervous system; DAM, diazomethane; DNA, deoxyribonucleic acid; eNOS, endothelial nitric oxide synthase; ERK, extracellular-signal-regulated kinases; H_2O_2, Hydrogen peroxide; HED, high energy diet; HFD, high fat diet; i.p., intraperitoneal; i.v., intravenous; IDDM, insulin dependent diabetes mellitus; IFN-γ, interferon-γ; IL-1, interleukin-1; iNOS, inducible nitric oxide synthase; LPL, lipoprotein lipase; MAPK, mitogen activated protein kinases; MIR-1, microRNA-1; MNU, n-methyl nitrosourea; NAD, nicotinamide adenine dinucleotide; NAD^{+}, oxidized nicotinamide adenine dinucleotide; NADH, reduced nicotinamide adenine dinucleotide; $NADP^{+}$, oxidized nicotinamide adenine dinucleotide phosphate; NADPH, reduced nicotinamide adenine dinucleotide phosphate; NF-κB, nuclear factor kappa beta; NGF, nerve growth factor; NIDDM, non-insulin-dependent diabetes mellitus; NO, nitric oxide; NOS, nitric oxide synthase; O_2^{-}, superoxide; O-GlcNAc, O-Linked β-N-acetylglucosamine; OH^{-}, hydroxyl radical; $ONOO^{-}$, peroxynitrite; p.o., per oral; PARP, poly (ADP-ribose) synthetase; PI3K, phosphoinositide 3-kinase; PIM-1, proto-oncogene serine/threonine-protein kinase; PKB, protein kinase B; PKC, protein kinase C; PNS, peripheral nervous system; RNS, reactive nitrogen species; ROS, reactive oxygen species; STZ, streptozotocin; TNF-α, tumor necrosis factor-α; XOD, xanthine oxidase.

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http://dx.doi.org/10.1016/j.cbi.2015.11.032

Animal model
Diabetic mortality
STZ toxicity

respect to age, body weight, gender and the target blood glucose level used to represent hyperglycemia. Therefore, protocol for STZ-induced diabetes in experimental animals must be meticulously planned. This review highlights specific skills and strategies involved in the execution of STZ-induced diabetes model. The present review aims to provide insight into diabetogenic mechanisms of STZ, specific toxicity of STZ with its significance and factors responsible for variations in diabetogenic effects of STZ. Further this review also addresses ways to minimize STZ-induced mortality, suggests methods to improve STZ-based experimental models and best utilize them for experimental studies purported to understand diabetes pathogenesis and preclinical evaluation of drugs.

1. Introduction

Streptozotocin (STZ) was first isolated from a soil microorganism *Streptomyces acromogenes* and showed broad spectrum antibiotic activity [1]. Since its discovery in 1959, STZ has been widely used for the induction of diabetes in experimental animals and in preclinical studies [2]. Among several available chemicals used to induce diabetes, STZ is most preferred to model human diabetes in animals. Structural, functional and biochemical alterations observed in STZ-induced diabetes resemble those which usually appear with diabetes in human. Therefore STZ-induced diabetes represents a clinically relevant model to study the pathogenesis of diabetes and associated complications in experimental animals [3]. Chemically, STZ is a glucosamine nitrosourea compound and named in the IUPAC system as (2-deoxy-D-glucose derivative of N-methyl-N-nitrosylurea). Structurally it resembles 2-deoxy-D-glucose but with a replacement at the C2 position with an N-methyl-N-group (Fig. 1) with a methyl group attached at one end and a glucose molecule at the another [2–6]. STZ is a hydrophilic compound occurring as a pale yellow or off-white crystalline powder with a molecular weight of 265 g/mol and molecular formula $C_8H_{15}N_3O_7$ [4,5]. It has a short biological half-life of only 5–15 min [2,3,7]. Accumulation of STZ is preferentially in pancreatic β-cells through the GLUT2 transporter system and results in β cell cytotoxicity [3,8]. Primarily it was used as an alkylating agent in the chemotherapy of metastatic pancreatic islet tumors [3]. The diabetogenic activity of STZ first became evident in 1963. Afterward it continued as an agent of choice to induce diabetes in experimental animals. STZ was found to cause extensive degradation of bacterial DNA in *Bacillus substilis* and revealed the possibility of β-cell DNA damage as a mechanism of diabetes induction in bacteria which is similar to the mechanism in mammals [1]. Being a glucose analogue, STZ enters β-cells via the GLUT2 transporter and accumulates intracellularly. STZ within the cells forms an alkylating agent, diazomethane (DAM), that causes DNA methylation and elicits diabetogenic action [4]. Apart from DNA methylation, STZ also induces diabetes via multiple mechanisms such as increased NADPH levels either by glucose auto-oxidation or diacylglycerol (DAG) production and increased O_2^- free radical generation, activation of protein kinase C pathway, glucose flux through the polyol metabolic pathway, accumulation of advanced glycation end products and cytokine secretion [9,10]. By involving all these mechanisms, STZ selectively damages β cells allowing STZ to be considered as a unique compound for modeling diabetes in animals with acceptable construct and face validity. However, reproducibility of this animal model is affected by factors such as variability in preparation and route of administration of STZ, dose of STZ, pharmacokinetics and pharmacodynamics across the species, animal strain, age and other characteristics of experimental subjects. Additionally, non-specific toxicity on other organs elicited by STZ also contributes to variability in its actions. STZ has been reported to induce both insulin-dependent (IDDM) and non-insulin-dependent diabetes mellitus (NIDDM) [2,11,12]. It is reported that the dose and duration of STZ plays an important role in induction of the type of diabetes. In experimental diabetes induction, knowing the different pathogenesis of IDDM and NIDDM in humans and different approaches of their treatment is one of the major concerns. IDDM (Type 1) is believed to be induced in experimental animals by a single STZ injection [13,14]. Whereas, NIDDM (type 2) has been shown to be induced by various approaches such as STZ injection following nicotinamide administration [15,16], high fat diet (HFD) feeding after low-dose STZ injection and STZ injection during the neonatal period [17–19].

In addition to the diabetogenic actions, STZ also possesses antitumor, anti-bacterial and mutagenic properties. A recent upsurge in reports on STZ-induced symptoms of Alzheimer's disease [20], chronic pain [21], genotoxicity [22], retinal neuron death [23], renal tumors [24] and bone deformities [25] in laboratory animals has rejuvenated interest of scientists to use STZ for experimental modeling of these pathologies. However, even though experienced scientists have expressed the cautious use of STZ, they have been skeptical about the successful induction of diabetes with STZ. In particular, the validity of STZ-induced diabetes protocol seems to be doubtful due to critical handling of STZ samples and variability in its effects. Usually, doses of STZ below 60 mg/kg induce a reversible rise in hyperglycemia whereas doses higher than 75 mg/kg in rats and 200 mg/kg in mice causes mortality in a dose dependent manner [26,27]. Consequently induction of a sustained diabetic state in rodents has been a challenge for every investigator working

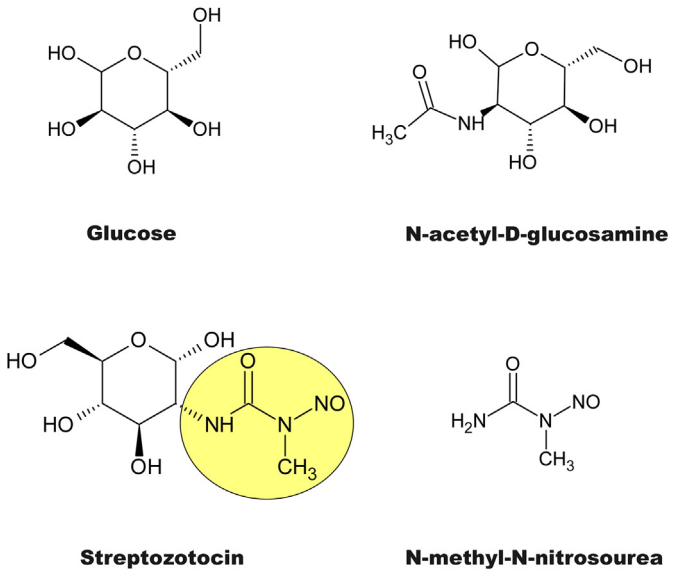

Fig. 1. Chemical structures of glucose, N-acetyl-D-glucosamine, streptozotocin and N-methyl-N-nitrosourea.