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STREPTOZOTOCIN INDUCED DIABETES: MECHANISMS OF INDUCTION

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ABSTRACT

Diabetes mellitus (DM) is major cause of mortality and morbidity worldwide. Streptozotocin (STZ) is one of the best compounds to induce experimental diabetes in animals. STZ after uptake in to the pancreatic beta (β)-cell splits into its glucose and methyl nitrosourea moiety. Due to its alkylation property, it further damages the DNA. Moreover, the DNA damage induces activation of Poly ADP ribosylation, an important process for diabetic activity of STZ. Furthermore, the polyADP ribosylation leads to depletion of cellular NAD⁺ and ATP. The depletion of the cellular energy stores occurs that ultimately results in pancreatic beta cell necrosis. In addition, it can be suggested that STZ is the agent of choice for reproducible induction of a diabetic metabolic state in experimental animals that may be attributed to its potent chemical properties. The present review unfalteringly thrashes out the mechanism of induction of DM in experimental animals.

Key words: Diabetes mellitus, Streptozotocin, Mechanism

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INTRODUCTION

DM has been considered as a group of heterogenous, hormonal and metabolic disorders characterized by hyperglycemia, glucosurea, polyuria, polydipsia and polyphagia [1,2]. In addition, DM is a chronic disorder which is associated with long-term complications including retinopathy, nephropathy, neuropathy and angiopathy [3,4]. Moreover, DM is regarded as a principle risk factor for various cardiovascular disorders, cerebral stroke and peripheral artery disease that results in increased mortality with

diabetics [5]. It has been comprehensively suggested that experimental animal models are one of the best strategies for the understanding of pathophysiology of any disease [6,7]. Numerous animal models have been developed for the past few decades for studying DM and testing anti-diabetic agents that include chemical, surgical and genetic manipulations [8,9]. One of the most potent methods to induce experimental DM is streptozotocin (STZ)-induced chemical induction of diabetes [10]. STZ acts as a DNA synthesis inhibitor in both bacterial and mammalian cells [11]. In bacterial

cells, a specific interaction with cytosine moieties leads to the degradation of the bacterial DNA, whereas, in mammalian cells, the DNA and chromosomal damage is done by mechanisms involving free radical generation during STZ metabolism. The induction of diabetes by STZ involves four different steps that ultimately produce necrosis of pancreatic β -cells [12,13]. Furthermore, various biological effects have been found to be associated with STZ that may be purely devoted to its hydrophilicity, glucose similarity and alkylation [14]. The present review critically explains about the phases of induction of DM by STZ. Moreover, the mechanism of diabetes induction alongwith the biological effects of STZ have been discussed in the review.

ETIOLOGY

STZ (2-deoxy-2-((methyl (nitroso)amino)carbonyl)amino)- β -D-glucopyranose) is a naturally occurring compound, formed by the bacterium *Streptomyces achromogenes*, which exhibits broad spectrum antibacterial properties [15,16]. STZ is a mixture of α - and β -stereoisomers that appear as a pale yellow crystalline powder. Moreover, STZ is extremely soluble in water, ketones and lower alcohols, whereas, slightly soluble in polar organic solvents. STZ has been formerly identified in the late 1950s as an antibiotic [15], while the molecular structure of STZ was first described by Herr et al. [17]. The drug was identified in a strain of the soil microbe *Streptomyces achromogenes* by scientists at the drug company Upjohn (now part of Pfizer) in Kalamazoo, Michigan. The sample of soil was taken from Blue Rapids, Kansas, which may be considered as the birthplace of STZ [18]. In addition, Upjohn filed for patent protection for the drug in August 1958 and was approved in March 1962. Moreover, in the mid-1960s, STZ has been noted to be the leading cause of selective necrosis of pancreatic β -islets, which suggested the role of STZ as an animal model of diabetes-induction in experimental animals [19]. Additionally, the National Cancer Institute (NCI) investigated STZ's use in cancer chemotherapy in the 1960s and 1970s [20].

PHASES OF DIABETES INDUCTION

STZ has been widely reported to induce experimental diabetes due to the selective destruction of the insulin-producing pancreatic β -islets [21]. Moreover, STZ induces a multiphasic blood glucose response in the experimental animals, accompanied by corresponding inverse changes in the plasma insulin concentration. Further, the reaction is followed by sequential ultrastructural β -cell changes leading to necrotic cell death [21,22]. The first phase, i.e., transient hypoglycemic phase, is not observed in response to STZ injection for the reason that STZ does not inhibit glucokinase. Further, the second phase starts with an increase in the blood glucose concentration, 1 h after administration of STZ which is followed by a consequent decrease in plasma insulin concentration. This first hyperglycemic phase, lasts for 2-4 hours, and is caused by the inhibition of insulin secretion leading to hypoinsulinemia [23]. Moreover, the β -cells show morphological characteristics like intracellular vacuolization, dilation of the rough endoplasmic reticulum, decreased Golgi area, reduced secretory granules and insulin content alongwith swollen mitochondria during this phase [24].

The third phase has been the hypoglycemic phase, which usually occurs 4-8 h after the injection of STZ and ends in several hours. It may be considered that a severe condition causes convulsions that may prove lethal without glucose injection, in a condition when the liver glycogen stores have been exhausted due to starvation [21]. This severe transition has been caused by the flooding of the circulation of insulin as the result of STZ-induced secretory granules and cell membrane rupture. In addition, the morphological changes comprises of the rupture of other subcellular organelles regarding cisternae of endoplasmic reticulum and the golgi complex. Moreover, mitochondria loose their structural integrity of the outer and inner cell membrane in this phase [25]. Furthermore, the fourth phase has been the permanent diabetic hyperglycemic phase.

According to the ultrastructural analyses, a complete degranulation of the pancreatic β -cells occurs. This phase is seen within 12-48 hours during which phase. the α -cells, other endocrine and non-endocrine islet cells, extrapancreatic parenchymal cells alongwith other non- β cells, remain undamaged [24].

MECHANISM OF ACTION OF STZ

STZ-induced diabetes has been commonly employed as an experimental model of insulin dependent DM. The mechanism of STZ action has been thoroughly studied that can be characterized quite well. STZ is a nitrosourea analogue, the toxic action of which requires its uptake into the cells [22]. The nitrosourea moiety is usually lipophilic, and hence, is rapidly uptaken by the cell membrane, whereas, the STZ is less lipophilic because of the hexose substitution. Moreover, STZ particularly binds with pancreatic β -cells by a low affinity glucose transporter (GLUT₂) in the plasma membrane [26,27]. In support, the cells not containing GLUT₂ transporter are more resistant to STZ action [28]. Further, the importance of GLUT₂ transporter in this process is considered because of toxicity of STZ which damages other body organs expressing this transporter, which includes kidney and liver. Moreover, STZ after uptake into the pancreatic β -cells, splits into its glucose and methylnitrosourea moiety [21]. Due to its alkylation property, it has been noted to cause DNA damage, which further induces activation of poly-ADP ribose polymerase (PARP), a process important for the diabetic activity of STZ. Furthermore, the poly ADP ribose leads to depletion of cellular NAD⁺ and ATP which further leads to the depletion of cellular energy stores ultimately leading to the β -cell necrosis [22]. In addition, STZ is the agent of choice due to its chemical properties, for the reproducible induction of a diabetic metabolic state in experimental animals.

BIOLOGICAL EFFECTS OF STZ

The biological effects of STZ may be attributed to its hydrophilicity, glucose similarity and alkylation. The attachment of the methylnitrosourea moiety

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of STZ to the 2 carbon of glucose as a carrier molecule results in the selectively accumulation of STZ in the pancreatic β -cells [29]. This inherent property of STZ makes it as a specific β -cell toxic and a potent diabetogenic compound [30]. The selective pancreatic β -cell toxicity of STZ alongwith the resulting diabetic metabolic state clearly relates to the glucose moiety in its chemical structure that enables STZ to enter the β -cells [31]. Moreover, the effect of STZ on glucose and insulin balance shows toxin-induced difficulties in its pancreatic β -cell function. In addition, the protein and mitochondrial DNA alkylation which results in the depletion of the NAD⁺ is responsible for the inhibition of insulin synthesis and secretion [22]. It has been noticed that nicotinamide, a PARP inhibitor, prevents early inhibition of β -cell function during the first day after STZ exposure, whereas, long term inhibition of insulin secretion six day after STZ exposure was not responded by PARP inhibitor. Moreover, the role of alkylation in pancreatic β -cell depletion has also been inspected by use of ethylating agents having less toxicity than their methylating counterparts [32]. It has been noticed that the N-ethyl-N-nitrosourea and ethyl methanesulphonate are significantly less toxic to insulin-producing cells, which showed that the mechanism of toxic action of STZ occurs due to alkylation [32,33]. It has also been noted that the initial circumscribed functional defects turn into more severe functional deficiencies when exposed to high cytotoxic STZ concentrations [34]. Furthermore, it has been documented that the toxicity of STZ resides in its ability to alkylate the biological macromolecules. It has been suggested that the toxic activity of STZ may be attributed to the DNA damaging effects which ultimately leads to the necrosis of the pancreatic β -cells via depletion of cellular energy stores [35,36].

CONCLUSION

The chemical induction of diabetes has been regarded as the most popular procedure for experimental induction of DM. The leading drug-induced diabetic model is the STZ-induced diabetes that is capable of inducing both type I and II DM in

experimental animals. However, surgical and genetic methods of diabetes induction have been allied with high degree of morbidity and mortality of experimental animals, thus, STZ-induced diabetes model appears to be the most reliable and easily reproducible method. Hence, more studies should be designed in order to completely apply the model of STZ-induced DM in experimental animals.

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