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Streptozotocin

by

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Abbreviations:
AD, Alzheimer's disease
ALX, alloxan
DMPO, 5,5-dimethyl-pyrroline-l-oxide
ESR, electron spin resonance
GSH, glutathione
MDA, malondialdehyde
MNU, methylnitrosourea
NAD^+, nicotinamide adenine dinucleotide
$NADP^+$, nicotinamide adenine dinucleotide phosphate
NMMA, L-N^G-monomethyl-arginine
$^{\bullet}NO$, nitric oxide
NOS, nitric oxide synthase
OFRs, oxygen free radicals
STZ, streptozotocin

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Abstract

Streptozotocin (STZ) is a glucosamine-nitrosourea compound that shows selective cytotoxicity to pancreatic β cells. It is used as an agent to induce experimental animal diabetes. The mechanisms of the induction of diabetes are not clear, but it is proposed they be related to the generation of free radicals, such as superoxide ($O_2^{\bullet-}$), hydrogen peroxide (H_2O_2) and nitric oxide ($^{\bullet}NO$), which result in DNA fragmentation. Intracerebroventricular injections of streptozotocin in animals can produce oxidative stress in the brain and cognitive impairment that may be relevant to sporadic Alzhiemer's disease. This paper briefly reviews STZ-induced animal disease models and the mechanisms that are related to free radicals.

Introduction

Streptozotocin, derived from a fermentation broth of *Streptomyces achromogenes*, was first isolated as a new antibiotic in 1956, which had a significant antimicrobial action for a wide spectrum of organisms [1]. In 1960 and 1961, antitumor activity screening at both Upjohn Research Laboratories and contract laboratories of the National Cancer Institute demonstrated antitumor effect, particularly in L1210 leukemia [2]. Murine tumor studies led to the discovery that STZ produced hyperglycemia [3], and further toxicology studies in dogs and rhesus monkeys demonstrated that STZ had a potent diabetogenic effect [4]. Ever since then, STZ has been used as a diabetogenic agent in experimental animals. The mechanisms of STZ-induced hyperglycemia are considered as follows: (1) STZ causes DNA strand breaks in pancreatic islets and stimulates nuclear poly(ADP- ribose) synthetase, and thus depletes the intracellular NAD^+ and $NADP^+$ levels, which inhibit proinsulin synthesis and induces diabetes [5]; (2) activated oxygen species, such as superoxide ($O_2^{\bullet -}$), hydrogen peroxide (H_2O_2), hydroxyl radical ($^{\bullet}OH$) and singlet oxygen ($^1O_2$), have been implicated to play important roles in diabetes, especially diabetic angiopathy [6]. Most recent research demonstrated that intracerebroventricular injection of STZ in rats produces oxidative stress in brain to cause cognitive impairment in rats and is likened to sporadic Alzheimer's disease (AD) in humans. This paper will focus mainly on reviewing the free radicals generated by STZ and the related animal models of STZ-induced diseases.

Structure and chemical properties of STZ

Streptozotocin, a glucosamine-nitrosourea compound, has a chemical name of 2-deoxy-2-(3-methyl-3-nitrosoureido)-D-glucopyranose($C_8H_{15}N_3O_7$). The structure is composed of a nitrosourea moiety with a methyl group attached at one end and a glucose molecule at the other as shown in Figure 1. The molecular weight is 265 g/mol [7].

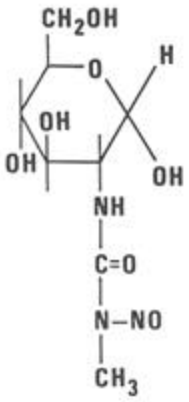

Figure 1. Structure of streptozotocin [7].

STZ structure has been determined to be the nitrosamide methylnitrosourea (MNU) linked to the C2 position of D-glucose. The nitrosamide MNU contributes to its alkylation properties and the glucose moiety directs it to the β-cell specifically. Once inside the cell, STZ is metabolized to cut apart between the 2'-carbon and the methyl nitrogen, the N-nitrosoureido moiety can do further damage in cells (Figure 2) [8].

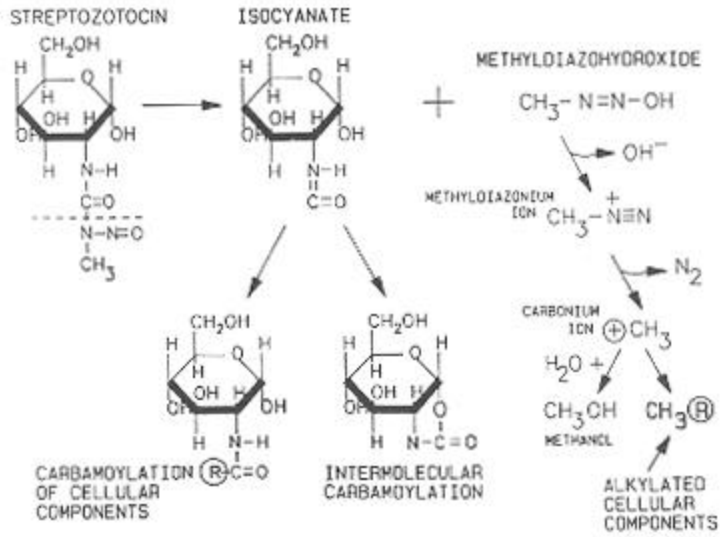

Figure 2. Spontaneous decomposition of STZ to form carbamoylating and alkylating species. The isocyanate component is able to either cabamoylate various cellular components or undergo intramolecular carbamoylation. The methyldiazohydroxide decomposes further to form a highly reactive carbonium ion or methyl radical, which is able to alkylate various cellular components such as DNA, protein or to react with H_2O to form methanol which can subsequently enter the 1-carbon pool. ® can be biological molecules. Adapted from [8].

Free radicals and STZ-induced diabetes

STZ administration damages pancreatic β cells and results in diabetes in experimental animals. The mechanisms by which STZ induces diabetes are not clearly understood. However, studies show that free radicals generated by STZ might be involved in the toxic action of STZ.

1. Oxygen free radicals (OFRs)

STZ is a chemically unstable molecule that accumulates in pancreatic β cells and produce toxic radicals during its decay. Studies using ESR spin-trapping techniques focused on the relationship between the effect of STZ on pancreatic β cells and free radicals formation by these cells. The results showed that STZ enhanced generation of the DMPO-OH radical adduct, which is a degradation product of the $O_2^{\bullet -}$ both in the presence and in the absence of cellular components in a hypoxanthine-xanthine oxidase (XOD) system with a homogenate of β cells (Figure 3) [9]. It is proposed that the cytotoxic effect of STZ be closely related to free radical generation in pancreatic β cells.

Figure 3. ESR spectrum of the DMPO-OH radical adduct obtained with a mixture of hypoxanthine and a homogenate of pancreatic β-cells. Adapted from [9].

STZ can also induce generation of H_2O_2, which may cause DNA fragmentation. When STZ was injected into rats, it accumulated in the islets. Administration of STZ to rats

in vivo stimulated H_2O_2 as shown in Figure 4 [10]. However, how H_2O_2 generated remains unknown.

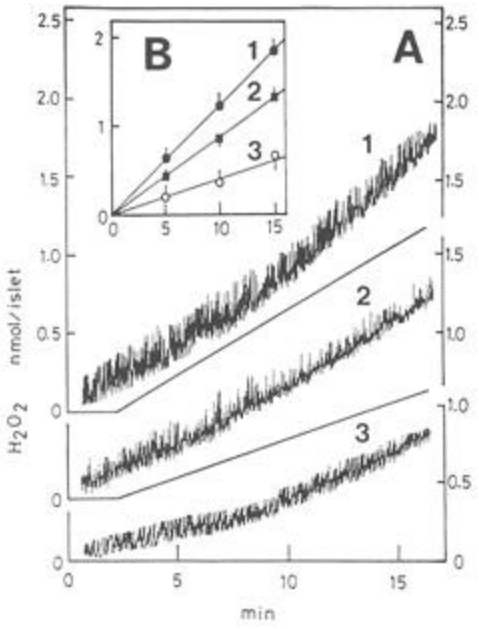

Figure 4. Effects of administration of STZ or ALX *in vivo* on H_2O_2 generation. A, B: 65 mg/kg i.v. STZ (1) or 65 mg/kg i.v. ALX (2) was injected into rats. Twenty minutes after STZ or ALX injection, islets were isolated from pancreas, and H_2O_2 generation was measured. Injection of STZ (1) or ALX (2) increased rate of H_2O_2 generation, which was higher than control rate (3). One typical experiment is shown in A, and data form 5-7 different experiments are shown in B. Adapted from [10].

The effect of STZ on DNA fragmentation *in vitro* was examined. Islets were incubated with STZ for 7-20 min, and velocity sedimentation of DNA was measured in an alkaline sucrose density gradient (Figure 5) [10].

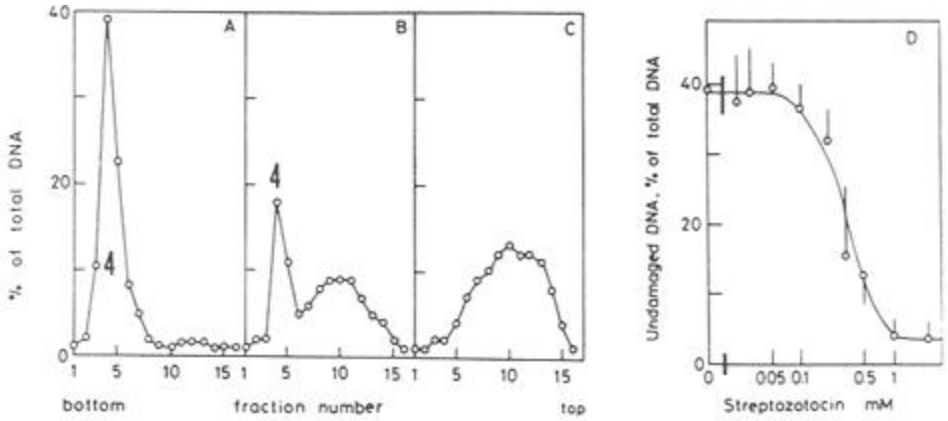

Figure 5. Effect of STZ on DNA fragmentation *in vitro*. Adapted from [10].

DNA of islets incubated without STZ for 20 min was recovered as a single peak near the bottom of the gradient (*4*, fraction 4), the position at which undamaged DNA sediments (Figure 5A). However, after only a 7-min incubation with 1 mM STZ, a considerable

amount of DNA sedimented as a broad peak in the middle of the gradient with a concomitant decrease in undamaged DNA (Figure 5B). After incubation with 1 mM STZ for 20 min, the DNA was almost completely fragmented (Figure 5C); the islet DNA sedimented slower as a broad peak, indicating that STZ induces islet DNA fragmentation. Graded doses of STZ induced DNA fragmentation, and their effects were observed at a concentration of 0.1 mM and were maximal at 1 mM (Figure 5D) [10]. These findings may support a proposal that STZ induces diabetes through the following biochemical events: STZ $\rightarrow$ H_2O_2 generation $\rightarrow$ DNA fragmentation $\rightarrow$ β-cell destruction.

2. Nitric oxide ($^{\bullet}$NO) generated by STZ

Nitric oxide generated by STZ has been proposed to be involved in the damage of pancreatic β cells. STZ consists of a 2-deoxyglucose substituted by N-methyl-N-nitrosourea at C2. The 2-deoxyglucose moiety acts as a carrier for the N-methyl-N-nitrosourea, which can decompose to generate $^{\bullet}$NO. Research showed that STZ could produce $^{\bullet}$NO by photo-decomposition or in acidic conditions [11]. Nitric oxide synthase (NOS) inhibitors, such as L-N^G-monomethyl-arginine (NMMA) and aminoguanidine reduce STZ-induced islet destruction and hyperglycemia in mice [12]. STZ-induced double-strand DNA breaks in rat pancreatic islets have been demonstrated to be inhibited by NMMA and nicotinamide, which suggest the involvement of STZ in NOS expression during β–cell injury [13].

Free radicals and STZ-induced Alzheimer's disease (AD)

STZ, when injected intracerebroventricularly in a subdiabetogenic dose in rat, has been found to cause prolonged impairment of brain glucose and energy metabolism. This is accompanied by impairment in learning and memory in addition to decreased choline acetyltransferase levels in the hippocampus [14, 15]. This model may be relevant to that of

sporadic AD in humans as both are characterized by a progressive deterioration of both cognitive functions and of cerebral glucose and energy metabolism.

Most recently, studies were undertaken to investigate the effect of intracerebroventricularly STZ on parameters of oxidative stress [16]. The levels of malondialdehyde (MDA), the end product of lipid peroxidation and glutathione (GSH) were measured. There was a gradual increase in levels of MDA in the brain of STZ-treated rats and a simultaneous decrease in the GSH level. The increase in levels of MDA and decrease in levels of GSH parallels with the diminution of learning and memory in STZ-treated rats proving that the STZ-induced learning and memory impairment is associated with oxidative stress in rats. It has been suggested that this model might be appropriate for investigations of antioxidants in Alzheimer's dementia.

Summary

Streptozotocin, a compound originally found to be as an antibiotic, has been widely used for induction of experimental diabetes mellitus. One of the most possible mechanisms that STZ cause animal diabetes is related to the generation of free radicals, including the generation of $O_2^{\bullet -}$, H_2O_2 and $^{\bullet}NO$, which can cause DNA fragmentation in cells. Most recently STZ was used to cause AD in experimental animals, and the mechanism of STZ-induced AD is also related to the generation of free radicals. An understanding of the mechanisms of the action of STZ as an agent to cause diabetes and AD is important for elucidating the causes of those diseases.

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