This student paper was written as an assignment in the graduate course

Free Radicals in Biology and Medicine

(77:222, Spring 2003)

offered by the

Free Radical and Radiation Biology Program
B-180 Med Labs
The University of Iowa
Iowa City, IA 52242-1181
Spring 2003 Term

Instructors:
GARRY R. BUETTNER, Ph.D.
LARRY W. OBERLEY, Ph.D.

with guest lectures from:
Drs. Freya Q . Schafer, Douglas R. Spitz, and Frederick E. Domann

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Streptozocin

by

Matt Zimmerman

1-251 BSB
Anatomy and Cell Biology
The University of Iowa
Iowa City, IA 52242-1109

For 77:222, Spring 2003

7. April 2003

Abbreviations

Inducible nitric oxide synthase	(iNOS)
L-N-monomethyl-arginine	(L-NMMA)
Nicotinamide adenine dinucleotide	(NAD)
1,10-phenathroline	(PNT)
Streptozocin	(STZ)
Superoxide Dismutase	(SOD)

Table of Contents

Abstract

Streptozocin (STZ) is a glucosamine-nitrosurea compound that was initially described as having antibiotic activity. However, more recent studies have identified STZ as a potent diabetogenic agent that causes diabetes mellitus in laboratory animals by destroying insulin-producing pancreatic β-cells. The exact mechanism by which STZ damages β-cells remains to be determined; however, substantial evidence has demonstrated that STZ causes DNA and chromosomal damage. Two working hypotheses have been developed to explain the cytotoxicity of STZ: 1) STZ spontaneously decomposes to produce reactive methylcarbonium ions that alkylate DNA; 2) STZ induces free radical generation including superoxide, hydroxyl radical, and nitric oxide, which cause DNA and chromosomal damage.

Introduction

Streptozocin (STZ) is a monofunctional nitrosourea compound that has been used in clinical trial for the past four decades. Streptozocin, isolated from the fermentation of *Streptomyces achromogenes,* was initially described as an antimicrobial compound that could act on a wide range of organisms [1]. However, subsequent studies in rats and dogs demonstrated that when STZ was administered intravenously it caused hyperglycemia; thus, STZ was deemed too toxic to be used as an antimicrobial agent but these studies showed that STZ had a potent diabetogenic effect. Subsequently, STZ has been and continues to be used to induce diabetes in experimental animal models. STZ is a highly genotoxic alkylating agent, which can cause cellular damage including DNA strand breaks and will eventually lead to cell death [2]. It has recently been postulated that free radicals are involved in the generation of DNA and chromosome damaged by STZ. This review will summarize the chemistry of STZ, while focusing on the link between STZ and free radicals. In addition, the use of STZ in experimental animal models to study human disease will be addressed.

Streptozocin Chemistry

Streptozocin is a glucosamine-nitrosourea compound with a molecular structure similar to that of 2-deoxy-D-glucose with a replacement at C_2 with a *N*-methyl-*N*-nitrosourea group [3]. The nitrosourea moiety and methyl group are attached at one end and a glucose molecule on the opposite side (Figure 1) [3]. Once inside the cell, STZ is able to spontaneously decompose to form an isocyanate compound and a methyldiazohydroxide (Figure 2) [4]. The isocyanate can undergo intramolecular carbamolyation or can carbamoylate other cellular

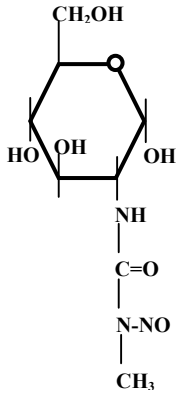

Figure 1. The structure of STZ demonstrates the N-methyl-N-nitrosourea moiety at C_2. Taken from [3].

components. The methyldiazohydroxide can decompose to form a highly reactive carbonium ion (Figure 2) [4], which is believed to be a key player in STZ-induced DNA alkylation causing interstrand DNA cross-links [2]. Structure-activity studies have demonstrated that the

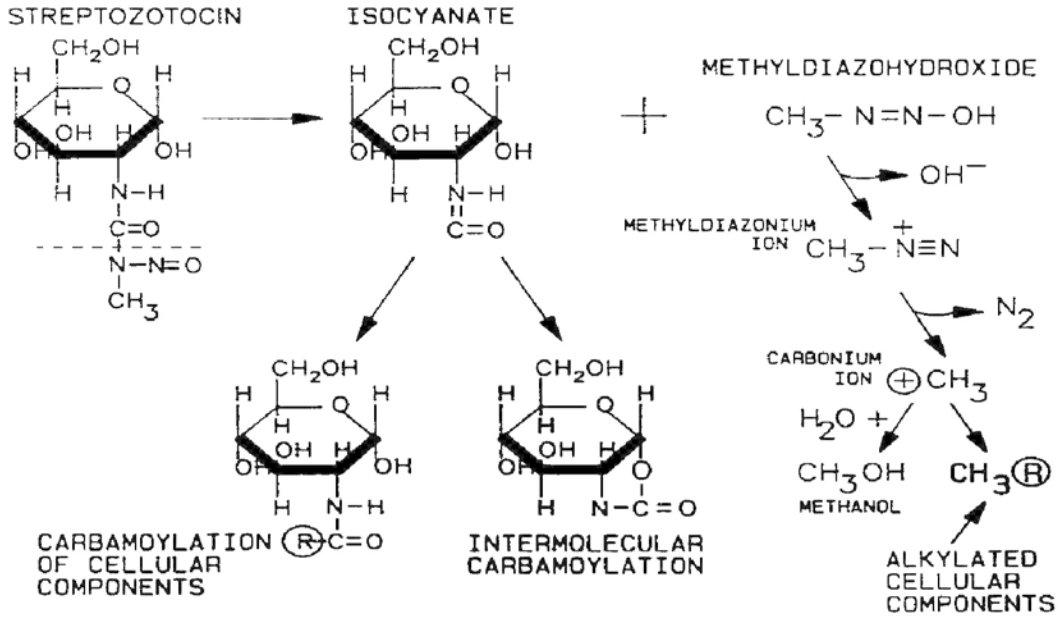

Figure 2. Once inside the β-cell, STZ can undergo spontaneous decomposition to from methylcarbonium ions, which can cause DNA damage through alkylation. Taken from [4].

methylnitrosourea metabolite has four times the alkylating activity of intact STZ, suggesting that the presence of the glucose moiety reduces the alkylating action of STZ [5]. The carbonium ions are highly reactive and can react with unshared pairs of electrons of the nitrogen and oxygen molecules located within the nucleophilic center of DNA [4]. Studies have provided evidence suggesting that the most predominant site for DNA alkylation is at the N^7 position and the O^6 position of guanine (Figure 3) [4]. The methylation at O^6 may interfere with hydrogen bonding and allow guanine to mispair with thymine, thus causing a point mutation [4]. In addition to the bases, STZ-mediated DNA

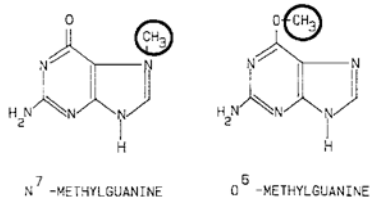

Figure 3. STZ damages DNA by causing the methylation of guanine at N^7 and O^6 position. Taken from [4].

alkylation can be targeted to the phosphate backbone resulting in the formation of phosphotriesters, which can cause conformational changes in DNA [4].

Streptozocin and Diabetes

Studies over the past 30 years have demonstrated that xenobiotics including STZ can cause a critical reduction in insulin secreting cells. Substantial evidence has shown that STZ induces diabetes mellitus in some laboratory animals, but it causes no more than mild, glucose intolerance in humans [2]. STZ is diabetogenic because it selectively destroys the insulin-producing beta cells by inducing necrosis. It is postulated that the selective beta-cell toxicity of STZ is related to the glucose moiety in its chemical structure (Figure 1), which enables STZ to enter the cell *via* the low affinity glucose transporter GLUT2 in the plasma membrane [6]. In fact, in animals the pancreatic islets cells can uptake STZ and its metabolite methylnitrosourea demonstrating that the sugar moiety is important for uptake in these cells [2]. It is hypothesized that the diabetogenic action of STZ in animals is mediated through a reduction of nicotinamide adenine dinucleotide (NAD) in pancreatic cells [2]. The DNA damage caused by STZ-mediated alkylation is repaired by an excision repair process, which requires the activation of the NAD-dependent enzyme poly (ADP-ribose) synthetase [4]. It is postulated that in the beta cell this enzyme is continuously activated, thus depleting the cell of NAD. The critical loss of NAD leads to a cessation of cellular function and eventually cell death (often referred to as the Okamoto model for beta cell damage) [4]. The evidence demonstrating that STZ-induced diabetes can be prevented by the administration of nicotinamide supports this hypothesis. Although elegant studies have demonstrated a role for NAD in STZ-induced DNA damage, an alternative hypothesis suggests that some of the diabetogenic properties of STZ could be related not to its alkylating properties but to its potential to generate reactive oxygen species.

Streptozocin and Free Radicals

In addition to STZ-induced cytotoxicity through DNA alkylation, recent studies have suggested that reactive oxygen species, including superoxide ($O_2^{\bullet-}$), hydrogen peroxide (H_2O_2), hydroxyl radical ($HO^{\bullet}$), and nitric oxide ($NO^{\bullet}$), play a critical role in the mechanism of DNA damage and cytotoxicity of STZ [3]. Initial studies demonstrated that STZ increased the generation $O_2^{\bullet-}$ in a hypoxanthine-xanthine oxidase system with a homogenate of pancreatic β cells [7]. These studies were extended in a cell-free system, which demonstrated that upon addition of STZ to the xanthine oxidase system $O_2^{\bullet-}$ generation increase in a concentration-dependent manner (Figure 4). In addition, STZ stimulates $HO^{\bullet}$ and H_2O_2 generation leading to DNA fragmentation in isolated rat pancreatic islet cells [3]. Reactive oxygen species can cause DNA damage by targeting the base or sugar. DNA single strand breaks are often the result of free radicals targeting the sugar resulting in damage to the phosphate backbone (Figure 5).

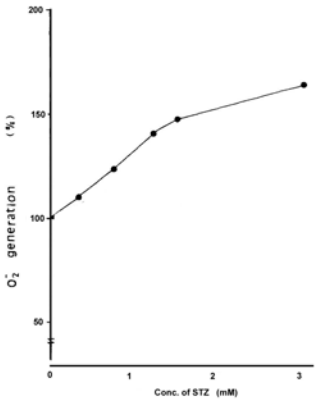

Figure 4. STZ induces superoxide generation from the xanthine oxidase system as measured by the generation of DMPO-$O_2^{\bullet-}$ adduct. Modified from Nukatsuka M, *et al*. [7].

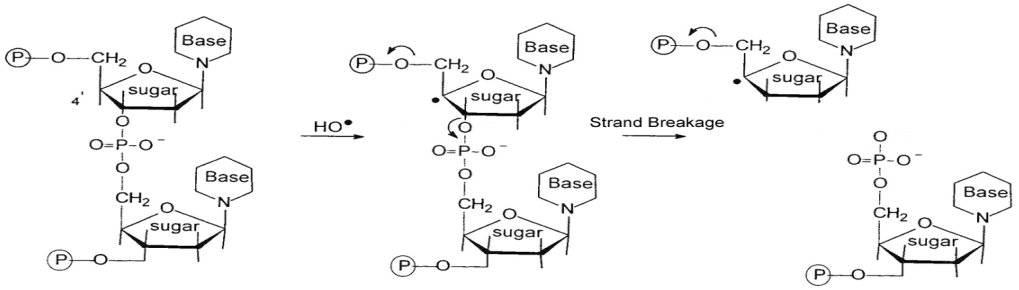

Figure 5. STZ can stimulate the production of free radicals in pancreatic beta cells and these free radicals, such as $HO^{\bullet}$ can damage DNA by targeting the sugar moiety of the molecule. This radical attack can lead to DNA strand breaks as seen in this schematic. Modified from course 77:222 notes written by Shafer, F.Q.

A collection of free radical scavengers including nicotinamide, catalase, superoxide dismutase (SOD), the $HO^{\bullet}$ scavenger dimethylurea, and α-tocopherol (Vitamin E) prevented the diabetogenic effects of STZ in experimental animal models [8]. In addition, SOD and catalase greatly attenuated the STZ-induced chromosome damage in Chinese hamster cells (CHO) and mosquito cells [8]. STZ-induced hyperglycemia in mice was prevented by L-N-monomethyl-arginine (L-NMMA) (Figure 6), an inhibitor of inducible nitric oxide synthase (iNOS); thus, suggesting a role for $NO^{\bullet}$ in STZ-induced diabetes mellitus [9]. In addition, histologically, the pancreas of mice treated with STZ and L-NMMA showed little or no cellular infiltration as well as significantly reduced destruction of islet cells. More recent studies contradict the source of STZ-stimulated $NO^{\bullet}$, suggesting that STZ spontaneously produces nitrite, which is oxidized to $NO^{\bullet}$ [10]. These results seem rational due to the fact that STZ does contain a nitrosourea moiety (Figure 1.) Further studies have demonstrated that $NO^{\bullet}$ is generated during the cellular metabolism of STZ and not by iNOS, as the formation of $NO^{\bullet}$ in hepatocytes in the presence of STZ was not blocked by NOS inhibitors [11]. However, the STZ-mediated DNA damage was significantly attenuated by the presence of an intracellular $NO^{\bullet}$ scavenger.

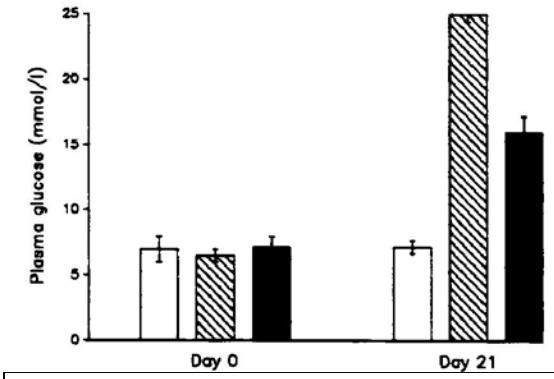

Figure 6. Nitric oxide plays an essential role in STZ-induced hyperglycemia in mice. Plasma glucose levels are similar between mice on the day they were treated with saline (empty bar), STZ (striped bar), or STZ plus L-NMMA (dark bar). However, STZ induced hyperglycemia 21 days after treatment and this was attenuated by treating mice additionally with L-NMMA. Taken from Lukic ML, *et al*. [9].

In addition to the above mentioned free radical scavengers, studies using metal chelators have begun to better understand the role of free radicals in the STZ cytotoxicity. One study used

the metal chelator 1,10-phenathroline (PNT), which enters the cell and, by forming a complex with iron, prevents the Fenton reaction (Reaction 1) from occurring, thus reducing the production

$$Fe^{2+} + H_2O_2 \rightarrow HO^{\bullet} + OH^{-} + Fe^{3+} \quad [1]$$

of $HO^{\bullet}$ [3]. PNT inhibited STZ-induced chromosomal aberrations; thus, indicating that the Fenton reaction is partially responsible for the production of chromosomal damage by STZ [3].

Summary and Conclusions

Streptozocin is a diabetogenic agent that is thought to damage pancreatic β-cells by causing DNA and chromosomal damage via alkylation and/or free radical generation. It is postulated, as shown by the Okamoto model (Figure 7), that the DNA damage causes a critical reduction in NAD, which leads to dysregulation of cellular functions and eventually cell death. While this hypothesis is generally accepted, it remains to be determined if the STZ-induced alkylation and free radical generation work together or independently to cause DNA damage. Due to these different chemical properties of STZ, further work is required to better understand the precise mechanism by which STZ destroys pancreatic cells and causes diabetes mellitus.

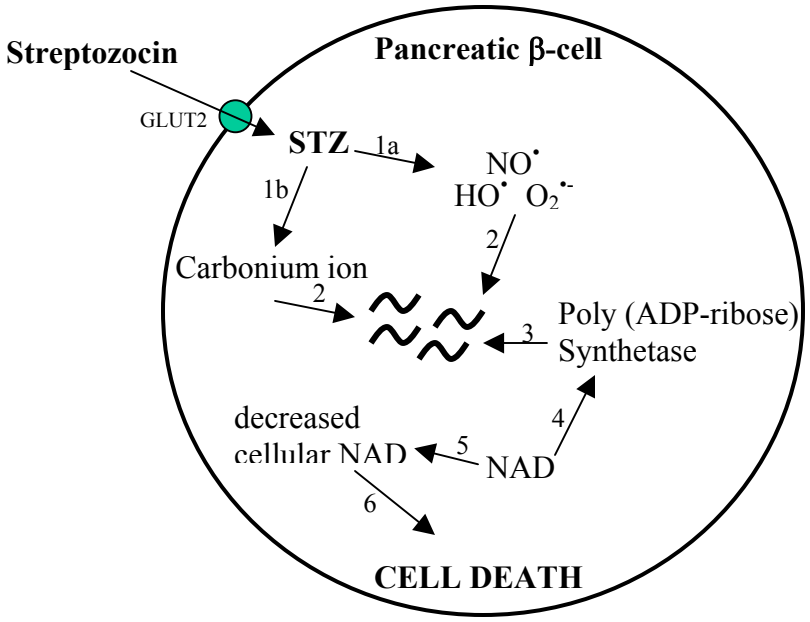

Figure 7. The Okamoto model hypothesizes that STZ causes DNA damage, which leads to a critical reduction of NAD levels thus causing cell death. The current proposal is that once inside the cell, STZ causes free radical generation (1a), which can cause DNA damage (2) or STZ decomposes to carbonium ion (1b). Carbonium ion can cause DNA damage *via* alkylation (2). The damaged DNA is repaired by activated poly (ADP-ribose) synthetase (3), which uses the cells source of NAD (4). The decreased levels of NAD (5) lead to cellular dysfunction and eventually cell death (6).

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