# 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**

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# Streptozocin

By

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For 77:222, Spring 2003

14. April 2003

Abbreviations:
BCNU, 1,3-*bis*(2-chloroethyl)-1-nitrosourea
CCNU, 1-(2-chloroethyl)3-cyclohexyl-1-nitrosourea
DHBA, dihydroxylbenzoic acid
DMPO, 5,5-Dimethylpyroline-N-oxide
ESR, electron spin resonance
MNU, N-methyl-N-nitrosourea
NAD, nicotinamide adenine dinucleotide
NOS, nitric oxide synthtase
SOD, superoxide dismutase
STZ, streptozocin
XO, xanthine oxidase

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Abstract:

Streptozocin (STZ) is an antibiotic, antineoplastic agent and a well-known diabetogenic agent that is used to induce diabetes under experimental conditions. It belongs to the chemical class of compounds known as nitrosoureas that contain a methyl nitrosourea moiety. This feature lends itself to the cytotoxic effects of STZ in pancreatic cells. The major target of STZ in the cell is DNA with the alkylation of bases. This DNA alteration could lead to chromosome instability whereby single and double strand breaks are apparent. Chromosome instability is a hallmark feature in some cancers in humans and STZ could promote carcinogenesis. One of the proposed mechanisms of toxicity for STZ is the induction of free radicals such as the hydroxyl radical and superoxide, and also nitric oxide produced during metabolism or decomposition. It is also thought that the free radical-induced toxicity of STZ is a limiting factor in its usefulness in chemotherapy.

Introduction:

Streptozocin (STZ), (CAS No. 18883-66-4), sometimes referred to as streptozotocin in older texts, was first isolated from a strain of *Streptomyces achromagenes* in 1967 [1]. It was originally identified as a broad-spectrum antibiotic with considerable activity against gram-positive and gram-negative microorganisms.

In addition to its antibiotic properties, STZ is a 2-deoxy-glucose derivative of the carcinogen N-methyl-N-nitrosourea (MNU) and belongs to a group of alkylating antineoplastic drugs known as alkylating nitrosoureas that include 1,3-*bis*(2-chloroethyl)-1-nitrosourea (BCNU), 1-(2-chloroethyl)3-cyclohexyl-1-nitrosourea (CCNU), fotemustine, clomesone and procarbazine. Most of these agents are clinically active against a broad range of tumor types including small cell lung cancer, mycosis fungoides, multiple myleoma, lymphomas and malignant melonma, but are not considered curative therapy due to limitations imposed by drug resistance [2].

The MNU moiety allows STZ to be taken up by the glucose transporter (GLUT-2) in the pancreatic β cells and selectively destroy them. The exact mechanism of STZ's toxicity is not fully understood, but it has been suggested that its primary effect on pancreatic β cells is DNA damage including alkylations and DNA strand breaks induced by free radicals generated when the compound decomposes inside the cell [2, 3]. This review will focus on the synthesis, toxicity and damage assessment of STZ.

Chemical Structure and Synthesis:

a. Chemical Structure:

The molecular structure of STZ was first described by Herr *et al.* [4] and is a 2-deoxy-D-glucose molecule substituted with a MNU group at $C_2$ (Figure 1). The chemical formulation of

STZ was elucidated using various techniques. The molecular weight analysis from isothermal distillations in water yields 269 Da and corresponds to the formula, $C_8H_{15}N_2O_7$. Titrations showed that STZ was neutral and had a strong maximum absorbance peak at 228 nm and a weak absorbance peak at 380 nm in the ultraviolet region. This suggested the presence of a nitro group that was predicted to elute when STZ was treated with a strong base (2N NaOH) to yield diazomethane [4]. The infrared spectrum of STZ indicates the presence of OH/NH groups and a band at 1705 $cm^{-1}$ was consistent with the presence of a carbonyl of the type $CON(NO)CH_3$ serving as the functional group at the $C_2$ position (Figure 1) that would produce the diazomethane upon treatment with alkali. The nuclear magnetic resonance spectrum of STZ, though not completely resolved, showed the presence of an $NH\text{-}CH_3$ group as a singlet 3 H at δ 3.15 and the absence of any $C\text{-}CH_3$ groups. The structure of STZ was confirmed by its synthesis.

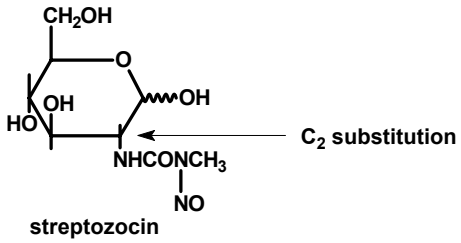


**Figure 1**. Structure of Streptozocin. Adapted from [4].

b. Synthesis Procedures:

Streptozocin has been synthesized by three different procedures. The first procedure uses tetra-O-acetyl-β-D-glucosamine hydrochloride as the starting material [4]. Treatment of tetra-O-acetyl-β-D-glucosamine hydrochloride with methyl isocynate ($CH_3NCO$) yields compound 1 that is further nitrosated with nitrosyl chloride (NOCl) to produce compound 2. Compound 2 is treated with ammonia ($NH_3$) to give STZ (Figure 2). This method of synthesis has a low yield due to several side reactions with the $NH_3$ treatment.

Due to the problems associated with the first procedure, a second improved synthesis that used D-glucosamine as a starting material was developed [5]. D-glucosamine is very abundant and provided a short reaction whereby it was reacted with N-nitromethyl carbonyl-azide ($CH_3NCON_3$) to produce STZ (Figure 2). The chemical yield increased slightly to 31%, but the method was plagued with the difficulty of producing the azide to be used in the reaction.

The third procedure is also a two step process that used D-glucosamine as the starting material [6]. It was reacted with methyl isocynate ($CH_3NCO$) to give compound 3, which was then nitrosated with nitrous acid ($HNO_2$) to produce STZ (Figure 2). This procedure was efficient in producing a chemical yield of 77-80%.

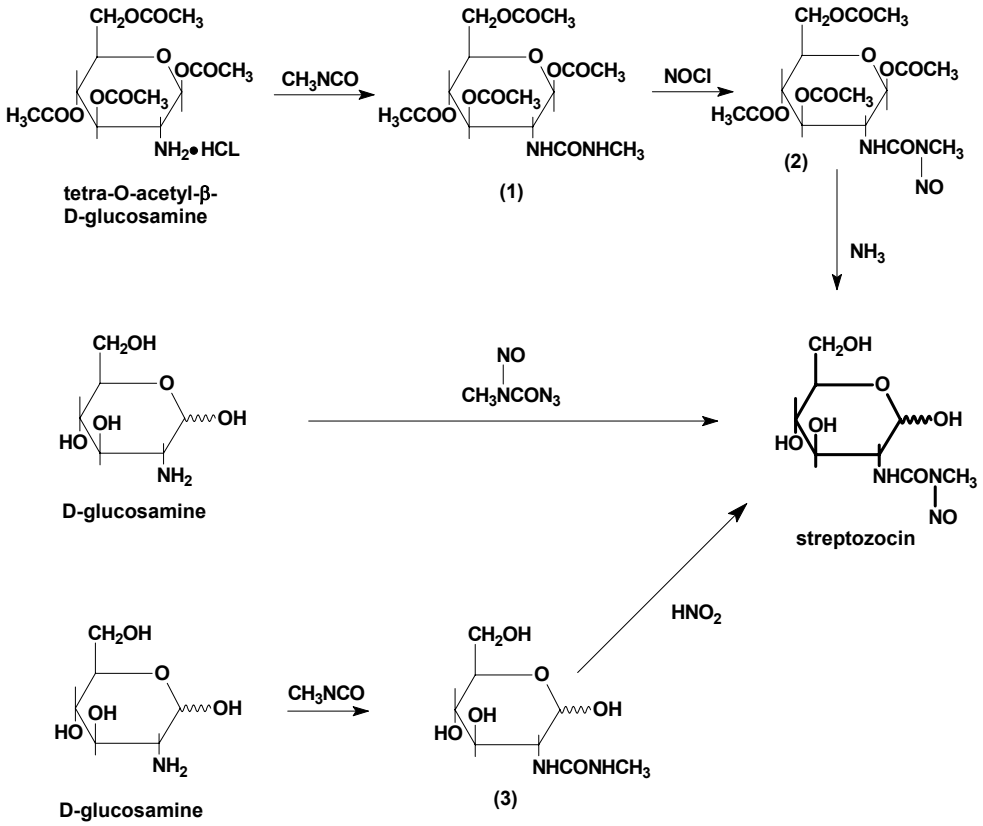


**Figure 2**. Synthesis approaches for streptozocin. Adapted from [1].

Mechanisms of Toxicity

Streptozocin has been used in animal models to induce diabetes mellitus a condition also known as hyperglycemia. It is thought to cause DNA strand breaks in the β-islet cells of the pancreas depleting the nicotinamide adenine dinucleotide (NAD) levels in the cell. The NAD depletion inhibits proinsulin synthesis and induces diabetes [3].

It is we11 established that DNA is the primary target of STZ toxicity [2-3]. There are two proposed mechanisms by which STZ is thought to impose toxicity on pancreatic β-islet cells with the model depicted in Figure 3 [3]. The first mechanism, DNA modification, implies that STZ induces free radicals that in turn damage DNA. The second mechanism, DNA methylation, suggests that STZ causes direct alkylation of DNA by reactive methylcarbonium ions ($^{\bullet}CH_3$, $CH_3^+$) formed during decomposition.

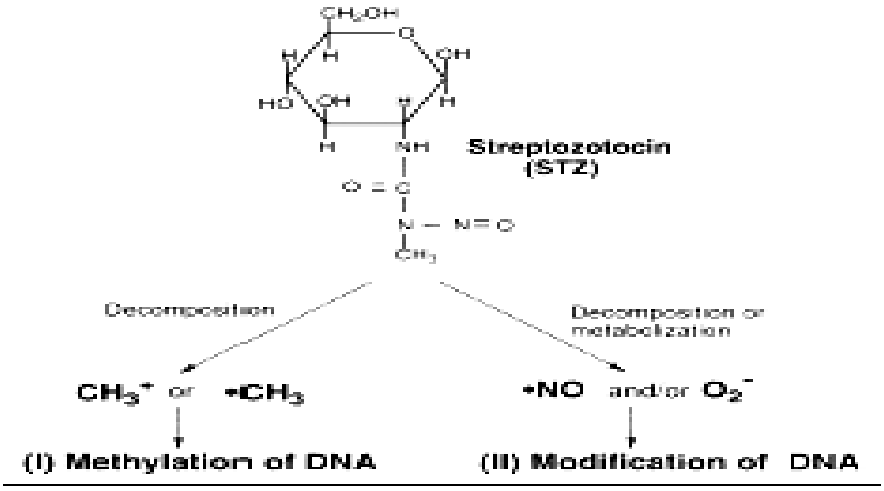


**Figure 3.** Proposed mechanisms of toxicity induced by streptozocin. Adapted from [3].

a. DNA Modifications:

Streptozocin has been shown to enhance the production of superoxide ($O_2^{\bullet-}$) in the xanthine oxidase (XO) system of pancreatic β-cells [7]. In this study, they were able to show that the addition of hypoxanthine in the presence of STZ produced the DMPO/$HO^{\bullet}$ radical

adduct ESR signal compared to no signal produced due to the DMPO/$O_2^{\bullet-}$ radical adduct. The DMPO/$HO^{\bullet}$ adduct is thought to be a decomposition product of the $O_2^{\bullet-}$ radical as the signal disappeared upon the addition of superoxide dismutase (SOD). The sugar moiety contained in the STZ molecular structure proved to be important for its free radical interactions since STZ derivatives actively enhanced the ESR in the XO system while MNU alone did not produce a signal.

Hydroxyl radical ($HO^{\bullet}$) formation was increased in animals treated with STZ as measured by trapping radicals with salicyclic acid as 2,3- and 2,5-dihydroxylbenzoic acids (DBHA) [8]. The increased $HO^{\bullet}$ levels correlated linearly with blood glucose levels and the concentration of STZ.

Nitric oxide (NO) generated during the cellular metabolization of STZ in the animal model provided evidence of its contribution to early DNA strand breaks [9]. In order to confirm that the NO formation was not attributed to nitric oxide synthtase (NOS), inhibitors of NOS used in the experiment did not block the NO formation generated in the presence of STZ.

b. <u>DNA Methylation:</u>

Streptozocin a potent alkylating agent induces damage to DNA by direct methylation. Highly electrophilic species such as $CH_3^+$ generated during STZ decomposition are expected to attack the most nucleophilic guanine sites on DNA [3]. In fact, reports indicate that STZ administration produces significant levels of $N^7$-methylguanine ($N^7$-MeG) and $O^6$-methylguanine ($O^6$-MeG) DNA adducts in the animal model [2]. The $N^7$-MeG is of greater biological significance as $O^6$-MeG adducts occur less frequently and are repaired by the $O^6$-alkylguanine DNA alkyltransferase.

Detection of STZ-Induced DNA Damage:

a. In Situ Nick Translation:

The nick translation reaction involves the incorporation of radioisotopes into single-stranded DNA breaks. It is used to assess DNA damage by measuring the radioactivity using an appropriate counter [10]. This method is a general technique and cannot be used to detect DNA damage in different cell types. On the other hand, *in-situ* nick translation demonstrates the specific localization of DNA damage in individual cells of the pancreas with immunostaining used to distinguish between α and β cells of the pancreas. Since STZ is known to induce DNA damage in pancreatic β cells, this method is very specific for measuring its effects.

Conclusion:

Streptozocin, a MNU derivative, was first isolated from *Streptomyces achromagenes.* It is the only naturally occurring compound in its class and possesses the unique properties of broad-spectrum antibiotic, and antitumor, carcinogenic, and diabetogenic agent.

Evidence suggests that DNA is the primary target of STZ induced toxicity and the biological effects are related to alkylation of specific sites on DNA bases and by free radicals generated during STZ metabolism. Both of these factors play a major role in the mechanism of DNA damage that essentially leads to the pancreatic cytotoxic effects seen in the animal models and humans.

The clinical application of STZ is related to its effect on pancreatic neoplasms, but drug resistance quickly develops thereby limiting treatment effectiveness. Further research into the mechanisms of STZ-induced toxicity may one day increase its antineoplastic qualities.

Acknowledgement: I would like to thank Dr. F. Schafer for her contributions to the chemical figures used in this paper and in previous papers.

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