

Review

Genotoxicity of Streptozotocin

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Abstract

Streptozotocin (Streptozocin, STZ, CAS No. 18883-66-4) is a monofunctional nitrosourea derivative isolated from *Streptomyces achromogenes*. It has broad spectrum antibiotic activity and antineoplastic properties and is often used to induce diabetes mellitus in experimental animals through its toxic effects on pancreatic β cells. STZ is a potent alkylating agent known to directly methylate DNA and is highly genotoxic, producing DNA strand breaks, alkali-labile sites, unscheduled DNA synthesis, DNA adducts, chromosomal aberrations, micronuclei, sister chromatid exchanges, and cell death. This antibiotic was found to be mutagenic in bacterial assays and eukaryotic cells. STZ is also carcinogenic; a single administration induces tumors in rat kidney, liver, and pancreas. Several lines of evidence indicate that free radicals are involved in the production of DNA and chromosome damage by this compound. Because of the use of STZ as an antineoplastic agent, the study of its genotoxicity has considerable practical significance. The purpose of this review is to present our current knowledge regarding the genotoxicity of STZ.

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1. Introduction

Streptozotocin (Streptozocin, STZ, CAS No. 18883-66-4) is a monofunctional nitrosourea derivative that was first isolated from *Streptomyces achromogenes* fermentation broth [1–3]. It also has been synthesized by three different procedures: (1) from tetra-*O*-acetyl glucosamine hydrochloride [3], (2) from D-glucosamine + *N*-nitrosomethyl carbamyl-azide [4], and (3) from D-glucosamine *N*-methylurea [5]. Its molecular structure, as described by Herr et al. [3] is shown in Fig. 1, and corresponds

to a 2-deoxy-D-glucose molecule substituted at C₂ with a *N*-methyl-*N*-nitrosourea group.

STZ is a member of a group of alkylating antineoplastic drugs known as alkyl nitrosoureas, which includes 1,3-bis(2-chloroethyl)-1-nitrosourea (BCNU); 1-(2-chloroethyl)-3-cyclohexyl-1-nitrosourea (CCNU); 1-(2-chloroethyl)-3-methylcyclohexyl-1-nitrosourea (meCCNU); 1-(4-arnino-2-methyl-5-pyrimidinyl)-methyl-3-(2-chloroethyl)-3-nitrosourea (ACNU), fotemustine, clomesone, and procarbazine, and that are clinically active against a broad range of tumor types, including small cell lung cancer, lymphomas, mycosis fungoides, multiple myeloma, glioma and malignant melanoma [6–10]. However, these agents are not considered curative therapy. One of the most important contributing factors to treatment failure and disease relapse is the development

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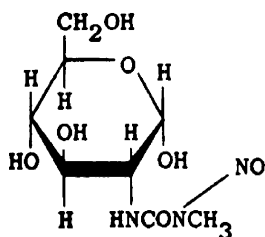


Fig. 1. Structure of Streptozotocin (STZ) as determined by Herr et al. [3].

of resistance to alkylating compounds [10]. In effect, it has been demonstrated that there are mammalian cells, including human cancer cells, which are highly or even completely resistant to the genotoxic effects of STZ and other methylating compounds [11–16]. Besides, the effectiveness of alkylating drugs in cancer therapy is often compromised by myelosuppression and a significant risk of therapy-related secondary malignancies [10,17,18]. In particular, STZ has been used alone or in combination with other chemotherapeutic drugs (vincristine, 5-fluorouracil, methyl-CCNU, procarbazine and 6-thioguanine) for the treatment of colorectal carcinomas and other gastrointestinal cancers, but severe toxicity and myelosuppression were observed in most of the patients [19–23], for earlier references see ref. [24] and the antitumor activity exhibited by STZ was very limited. However, a recent report suggests that a combination of STZ with doxorubicin could be used for the clinical treatment of pulmonary chemodectomas [25].

STZ has broad spectrum antibiotic activity [1,2] and is often used to induce diabetes mellitus in experimental animals through its toxic effects on pancreatic β cells [26,27]. Besides its antibiotic and diabetogenic properties, STZ is genotoxic in a variety of assays, including microbial mutagenesis, unscheduled DNA synthesis, micronucleus, chromosomal aberrations (CAs) and sister chromatid exchanges (SCEs).

The most recent genotoxicity review articles about STZ were written about 20 years ago [24,28,29]. During the past two decades, some important findings about the genotoxic effects of STZ were made. The purpose of this review is to present our current knowledge regarding the genotoxicity of STZ.

2. DNA damage by Streptozotocin

2.1. Types of DNA lesions and mechanism of DNA damage induced by STZ

STZ is a potent alkylating agent known to directly methylates DNA [29–31]. In effect, STZ administration has been reported to produce significant levels of *N*⁷-methylguanine (*N*⁷-MeG), *O*⁶-methylguanine (*O*⁶-MeG), *N*⁷-methyladenine, and *N*³-methyladenine DNA adducts in rat kidney [30]. A significant fraction of the lesions are repaired by *O*⁶-alkylguanine DNA alkyltransferase in a process that does not involve excision repair or an abasic (alkali-labile) site [32]. The base adduct of principal biological significance is *N*⁷-MeG, since more than 70% of DNA methylation occurs at the *N*⁷ position of guanine [30,33–36]. Moreover, although the *O*⁶-MeG adducts and other less common methylated bases such as *O*⁴- and *O*²-methylthymidine may be more significant promutagenic lesions than *N*⁷-MeG [37], the latter may also lead to mutations since apurinic sites are promutagenic due to preferential incorporation of adenine by DNA polymerases at abasic sites [38]. Thus, *N*⁷-MeG serves as an indicator of overall DNA damage by STZ.

In addition to covalent adducts, STZ induces DNA strand breaks and alkali-labile sites as reported by Erickson et al. [39] in Chinese hamster V79 cells. Moreover, DNA cross-linking was observed with several nitrosoureas but not with STZ. Similarly, Petzold and Swenberg [40] showed that a single i.v. dose of STZ (50–100 mg/kg) induces strand breaks in kidney and liver of rats, whereas others [41–43] demonstrated that STZ induces DNA strand breaks in rat pancreatic islets. More recently, LeDoux et al. [44] demonstrated the induction of DNA strand breaks and adducts (*N*⁷-MeG) by STZ in monolayer cultures of neonatal rat β cell, and Brambilla et al. [45] showed that a single p.o. dose of the antibiotic (20–180 mg/kg) induces DNA strand breaks and alkali-labile sites in rat liver.

Although STZ per se does not induce DNA inter-strand cross-links, studies in human cell lines of the Mer⁺ phenotype (i.e. proficient in the repair of guanine *O*⁶ alkylations) have shown that this antibiotic apparently saturates the monoadduct repair system and allows other alkylating drugs to form these lesions in the DNA molecule [46,47].

Induction and repair of DNA strand breaks and alkali-labile sites by STZ was analyzed by several authors. Mossman et al. [48] showed that STZ induces DNA single-strand breaks in a rat insulinoma cell line (RINr 38) and that these lesions can be repaired in a time-dependent manner, with most repair completed by 24 h post-exposure to the antibiotic. Using the same cell line, Pettepher et al. [49] demonstrated that STZ induces alkali-labile sites in a dose-dependent fashion within the mitochondrial DNA and that these lesions can be repaired. These authors found that 8 h after exposure to the antibiotic, 55% of the lesions induced in the mitochondrial DNA were removed. The level of repair increased to 70% after 24 h. In comparison, only 46% of N^7 -MeG adducts were removed across the entire cellular genome. In addition, Fram et al. [50] found that STZ caused single-stranded DNA breaks in *Escherichia coli* strains not proficient in recombinational repair which lack either RecA protein or RecBC gene products. DNA breaks in RecBC cells were repaired, while those present in RecA cells were not. These findings establish the critical importance of recombinational repair in ameliorating DNA damage caused by STZ in *E. coli*. More recently, STZ was shown to induce DNA strand breaks and mutations in liver and kidneys of mice dosed i.p. as determined by a microgel electrophoresis assay and a transgenic mouse mutation assay, respectively, at levels of 25–150 mg/kg [51].

Kraynak et al. [52] analyzed the extent and persistence of STZ-induced DNA damage and cell proliferation in rat kidney using in vivo alkaline elution and BrdUrd labeling assays, providing evidence that the cellular and molecular repair responses to a single diabetogenic dose of STZ are prolonged, requiring up to 3 weeks to complete. A dose of 2.5 mg/kg STZ was the lowest dose to induce detectable DNA strand breaks and extensive damage was produced by the diabetogenic dose of 60 mg/kg. Significant DNA damage was detected up to 14 days after dosing with return to near background levels by 20 days. Similarly, treatment with 60 mg/kg STZ was associated with increases in BrdUrd labeling indices 4 and 9 days after treatment with resolution by 27 days [52]. The mechanism of induction of DNA damage by STZ was recently investigated in vitro, using a human cell line and ^{32}P -labeled DNA fragments isolated from human genes [53]. It was found that STZ frequently initiates DNA modifi-

cation at guanines, especially at the middle guanine in runs of three and at the guanine at the 3'-end of runs of two guanines, similar to *N*-methyl-*N*-nitrosourea, a typical methylating agent. This suggests that STZ induces DNA damage by methylation of guanines via methyl cations.

Another genotoxic effect of STZ is the induction of unscheduled DNA synthesis (UDS), as reported by Tyson and Mirsalis [54] in isolated rat kidney cells following in vivo treatment with STZ and confirmed a few years later by Shepherd et al. [55], using cultured normal rat pancreatic epithelial cells. Induction of DNA damage by STZ is also evident by an increased expression of growth arrest and DNA damage-inducible genes *gadd* 153 and *gadd* 45 observed in rat pancreatic islets treated with the antibiotic [56].

On the other hand, studies in DNA repair-deficient *E. coli* mutants have shown that STZ inhibits DNA synthesis [50]. This effect was demonstrated in strains not proficient in recombinational repair (which lack either RecA protein or RecBC gene products) and in *E. coli* cells lacking both 3-methyladenine DNA glycosylases I (tag) and II (alkA). DNA synthesis inhibition was also demonstrated in Chinese hamster cells CHO-9 and V79 by Capucci et al. [36]. Induction of DNA repair synthesis by STZ was observed by Sandler and Swenne in the pancreatic islets of mice [57,58] and fetal rat [59]. However, DNA replication remained unaffected by STZ treatment. These results support the view that STZ acts on the β cell by causing DNA damage followed by DNA repair synthesis.

Besides the above mentioned effects on the DNA molecule, STZ induces cell death by apoptosis and necrosis. Cell death by apoptosis was observed in cultured pancreatic β cell HIT-T15 and RINm5F after treatment with STZ [60]. In addition, Saini et al. [61] showed that STZ at low doses induces apoptosis and at high doses causes necrosis in a murine pancreatic β -cell line, INS-1. Higher rates of apoptosis, as compared to necrosis, were observed when cells were exposed to 15 mM STZ for 1 h followed by a 24 h recovery period. Higher doses of STZ (30 mM) caused the cells to undergo necrosis (22%) as well as apoptosis (17%). Very recently, Harel et al. [62] examined the potential of known diabetogenic agents STZ and alloxan, for induction of direct injury in an immortal human keratinocyte

(HaCat) cell line. Cells treated with 10 and 20 mM STZ showed a significant increase in apoptosis (3.9- and 6.7-fold), but not in necrosis, compared to naive cells.

2.2. DNA damage and free radicals

Although it is generally accepted that the cytotoxicity produced by STZ depends on DNA alkylation [29,30], several lines of evidence indicate that free radicals play an essential role in the mechanism of DNA damage and cytotoxicity by STZ. First of all, it was found that STZ enhances O_2^- radical generation by the xanthine oxidase system of pancreatic β cells [63,64] and stimulates H_2O_2 generation and causes DNA fragmentation in isolated rat pancreatic islets [65]. These latter effects were evident at a concentration of 0.1 mM and were maximal at 1 mM of STZ. In addition, Ohkuwa et al. [66] demonstrated the generation of OH^- radicals by STZ in diabetic rats and Bedoya et al. [67] showed that the DNA damage (double strand breaks) induced by STZ (0.55 mM for 30 min before culture) in rat pancreatic islet cells can be prevented by *N*-monomethyl-arginine (which inhibits the inducible form of nitric oxide synthase) or by nicotinamide (a free radical scavenger). Recent evidence indicates that nitric oxide (NO) is also involved in the production of DNA damage by STZ. In effect, Kroncke et al. [68] demonstrated that NO generation during cellular metabolism of STZ contributes to rat pancreatic islet cell DNA damage. This NO formation is not due to a NO synthase (NOS) activity since NO formation in hepatocytes in the presence of STZ is not blocked by the NOS inhibitor NG-methyl-L-arginine. Furthermore, early DNA-strand breaks induced either by STZ or by the NO donor nitroprusside were both significantly reduced in the presence of an intracellular NO scavenger. Very recently, Chen et al. [69] found that the overexpression of metallothionein (an inducible antioxidant protein) in pancreatic β cell reduces the STZ-induced DNA damage and diabetes. Furthermore, a variety of free radical scavengers, including superoxide dismutase (SOD) [70,71], the artificial SOD, copper(II) (3,5-diisopropylsalicylate)₂ [72] the hydroxyl radical scavenger dimethylurea [73], and Vitamin E [74], were found to protect animals from the diabetogenic effects of STZ.

Despite all the above data, there are a few reports suggesting that free radicals may not be involved in the DNA damage by STZ. In effect, it has been reported that exogenous SOD or catalase (CAT) do not prevent the DNA breaks induced by STZ [41–43]. Moreover, in 1999, Murata et al. [53] found that scavengers for reactive oxygen species or NO did not inhibit the induction of DNA damage by STZ in a human cell line. On the other hand, damage induction was inhibited by sodium acetate and sodium chloride, which can reduce the reactivity of methylating agents to DNA via the sodium cation. The reasons for the above disagreements are not clear. They may result from the different experimental approaches employed. Nevertheless, most of the studies performed so far regarding the effect of free radical scavengers on the DNA damage and cytotoxicity induced by STZ clearly support the idea that the genotoxic action of this antibiotic is mediated by free radicals.

In summary, current knowledge about the genotoxic effects of STZ indicates that this compound induces DNA damage by alkylation of specific sites on DNA bases and that free radicals generated during STZ metabolism seems to play a significant role in the mechanism of DNA damage and cytotoxicity by STZ. DNA lesions produced by STZ includes double and single-strand breaks, covalent adducts and alkali-labile sites. Severe DNA damage by STZ results in cell death by apoptosis or necrosis. Furthermore, the DNA strand breaks resulting from the alkylating action of STZ can lead to chromosomal rearrangements.

3. Chromosomal effects

3.1. Induction of chromosome damage by STZ

Although there are early reports on the clastogenic effects of STZ in mammalian cells [75,76], it was not until a few years ago that the chromosomal effects of this antibiotic was intensively investigated. STZ was found to produce all types of aberrations in the first post-treatment mitosis (i.e. dicentrics, rings, deletions, exchanges, breaks and gaps) but with a clear predominance of chromatid-type aberrations in all of the systems studied. These systems included the use of Chinese hamster cells, mosquito cells and human lymphocytes [16,77–80]. Our recent study in human

lymphocytes [80] shows that STZ (1 mM, for 1 h) induces CAs (mainly chromatid-type) in cycling but not in unstimulated lymphocytes, indicating that this antibiotic does not induce CAs in the G₀ phase of the cell cycle. Surprisingly, no induction of CAs by STZ in isolated lymphocytes was detected, probably because of a lethal effect of this compound, as suggested by the marked depression of the mitotic index that was observed in some samples [80]. The fact that no metaphase cells were found in some chromosome preparations indicates that STZ is cytotoxic for human lymphocytes, producing an inhibition of mitosis. A similar effect was previously observed by Bhuyan in mouse leukemia L1210 cells [75,81].

It has been shown that agents that induce aberrations primarily because of O⁶-MeG will give a much larger increase in aberrations in the second metaphases after treatment than in the first [16,82,83], and that N-alkylations are the major source of aberrations observed in the first post-treatment mitosis [84]. Our study in human lymphocytes suggests that STZ does not exhibit a delayed clastogenic effect, since no induction of CAs was found in second division metaphases from 72 h cultures [80]. However, in our most recent work about the effects of STZ on human cells, we found that, under similar experimental conditions to those previously used with peripheral blood lymphocytes, this compound induces severe chromosome damage (fragmentation and pulverization) and has a delayed clastogenic effect in human colon cancer cells in vitro [85]. Therefore, the chromosomal sensitivity of human cells to STZ depends on the cell type.

The chromosomal effects of STZ were also studied using the micronucleus and SCEs assays. This antibiotic was found to be a good inducer of micronuclei in mouse bone marrow cells [86,87] and SCEs in mammalian and insect cells [14,16,77,79,80]. Human lymphocytes proved to be highly sensitive to the induction of SCEs by STZ [80]. Besides, STZ was found to produce a similar effect on the frequency of SCEs in lymphocytes obtained from whole blood cultures and purified lymphocytes, suggesting that blood components do not interfere in the SCEs response of these cells. Like S-dependent agents, STZ was found to induce SCEs at concentrations lower than those required for the induction of CAs [80]. Induction of CAs and SCEs in different Chinese hamster cell

lines were analyzed by Capucci et al. [16,77]. They found that STZ induces CAs and SCEs in CHO-9 cells and its mutant EM-C11 cell line (hypersensitive to alkylating agents), although the mutant cell line was found to be more sensitive than parental cells to the killing and CAs-inducing effects of the antibiotic [77]. Moreover, V79 cells were found to be more resistant than CHO-9 cells to the induction of CAs and SCEs by the antibiotic [16].

It has been recently shown that the clastogenicity of STZ in mouse bone marrow, as measured by the micronucleus assay, can be potentiated by prior treatment with the O⁶-alkylguanine-DNA-alkyltransferase inactivator O⁶-benzylguanine [87]. This effect was found to be significantly inhibited in myeloablated mice reconstituted with bone marrow expressing the O⁶-benzylguanine-resistant mutant of human O⁶-alkylguanine-DNA-alkyltransferase (hATPA/GA) as a result of retroviral gene transfer. This finding clearly indicates that O⁶-MeG lesion in DNA is clastogenic in vivo and establish O⁶-alkylguanine-DNA-alkyltransferase as a major protective mechanism operating against such damage. The potentiation of STZ-induced clastogenicity by O⁶-benzylguanine suggests that the clinical use of both compounds simultaneously, could substantially increase the secondary cancer risk. However, bone marrow cells could be protected from potentiation of the clastogenicity of STZ during chemotherapy by the use of hATPA/GA via gene therapy [87].

Despite the clastogenic capacity of STZ, it has been demonstrated that there are mammalian cells, including human cancer cells, which are highly or even completely resistant to the clastogenic and SCEs-inducing effect of STZ and other methylating compounds [11–16,83,84,88]. This resistance has been usually ascribed to the activity of the DNA repair protein methylguanine-DNA-methyltransferase (MGMT), which demethylates mainly O⁶-MeG residues from DNA molecule [10,15]. In fact, methylating agents like STZ were found to decrease MGMT levels by introducing O⁶-MeG residues in DNA which are then repaired by the MGMT protein [89–91]. By decreasing MGMT activity, STZ enhances the cytotoxicity of other alkylating compounds, like BCNU [92,93]. In human trials, STZ by single dose injection (500 mg/m²) reduced MGMT levels in lymphocytes by 40% and after three doses by 75% [94]. Based on

these studies, some clinical trials were initiated using a combination of STZ and BCNU [95–98]. However, these trials did not show much promise, probably because the degree to which MGMT is inactivated in tumor tissue is not adequate for enhancement of cross-link formation, as suggested by Dolan [10] and supported by Willson et al. [97] who demonstrated MGMT depletion in lymphocytes but not in metastatic colorectal cancer following administration of STZ.

Mammalian cells that express a high level of resistance to methylating compounds usually express detectable amounts of MGMT and are defined as Mer⁺ [11]. Conversely, mammalian cells that do not express MGMT activity are defined as Mer⁻. Human and rodent cells that do not express MGMT activity and are resistant to methylation have also been reported, suggesting that the cellular resistance of these cells to the cytotoxic effect of DNA methylation may be determined by processes other than MGMT activity [12,13,36,99,100]. Some hypotheses are currently being proposed concerning the resistance of mammalian cells that do not express MGMT activity (Mer⁻) to DNA-alkylation damage. One hypothesis is a possible defect or loss in the mismatch repair system, as suggested by Aquilina et al. [101], to explain the resistance of CHO clones to MNU-induced DNA damage. An alternative hypothesis for the resistance of mammalian cells to alkylation damage is the existence of a post-replication repair system [102]. However, a mismatch repair failure or a post-replication repair system are error-prone mechanisms, leading to an increase in the spontaneous and/or induced mutations, by the persistence of the pre-mutagenic lesion *O*⁶-MeG in the DNA, as demonstrated earlier [12,103]. Nevertheless, Capucci et al. [16] demonstrated that STZ is equally effective in inducing point mutations in the cell lines CHO-9 and V79, which are Mer⁻, despite the fact that V79 cells were found to be more resistant than CHO-9 cells to the induction of CAs and SCEs by the antibiotic. This indicates that an unknown error-free mechanism of tolerance to DNA methylation damage is active in V79 cells.

In summary, cytogenetic damage by STZ can be manifested as CAs, SCEs or micronuclei. All the data available indicates that, like S-dependent agents, STZ is an efficient inducer of SCEs. However, unlike agents with a true S-dependent action, STZ induces both chromosome- and chromatid-type

aberrations. Therefore, although it can be considered an S-dependent agent, STZ acts on chromosomes both in an S-dependent and S-independent manner.

3.2. Prevention of STZ-induced chromosome damage

3.2.1. Antioxidant compounds

The antioxidant enzymes SOD (which converts O₂⁻ into H₂O₂) and CAT (which scavenges H₂O₂) and the OH⁻ radical scavenger mannitol were found to prevent to a great extent the STZ-induced chromosome damage in CHO and mosquito cells [78]. Addition of liposome-encapsulated antioxidants produced a significant and dose-dependent decrease in the yield of STZ-induced clastogenesis in these cells. Accordingly, it was assumed that DNA damage by STZ partially depends on free radicals production and that the protective effect of SOD, CAT and mannitol is due to the scavenging of O₂⁻, H₂O₂, and OH⁻ radicals generated by the antibiotic.

3.2.2. Metal chelators

In order to gain further insights into a possible involvement of free radicals in the clastogenic action of STZ, we recently analyzed the effect of the metal chelating 1,10-phenanthroline (PNT) on the induction of CAs and SCEs by STZ in CHO and mosquito cells [79]. PNT is a compound that enters the cell and, by forming a complex with iron, prevents the Fenton reaction from occurring, thus blocking the production of the OH⁻ radical. [104]. Although Eizirik et al. [105] previously showed that PNT does not protect against the diabetogenic effects of STZ, we found that PNT inhibits to a great extent the yield of STZ-induced CAs but does not prevent the induction of SCEs in CHO and mosquito cells [79]. This fact indicates that intracellular transition metals are implicated in STZ-induced CAs but not SCEs, and that the Fenton reaction (Fe²⁺ + H₂O₂ → OH^{*} + OH⁻ + Fe³⁺) is partially responsible for the production of CAs by this compound.

Current hypothesis about the induction of CAs and SCEs by methylating agents points out that *O*⁶-MeG and *N*-methylated bases-induced damage occurs by mismatch repair which operates at sites of *O*⁶-MeG-T or by depurinations and base excision repair intermediates, respectively, which block DNA replication by

inhibiting chain elongation, leading to double strand breaks and exchanges (see ref. [106] for a review). The evidence available suggests that, in the case of STZ, the induction of CAs also involves the generation of free radicals by the antibiotic in the presence of transition metal ions.

Continued studies on the clastogenic action of STZ coupled with molecular analysis of the mechanisms underlying the induction of DNA and chromosome damage by this compound will certainly enhance our understanding of the genotoxicity of this antibiotic.

4. Mutagenesis

4.1. Bacterial assays

A number of studies have shown that STZ is positive in all of the Ames tester strains [86,107–112]. The mutagenicity of STZ was tested in the base-pair substitution *Salmonella typhimurium* mutant hisG46 and the hisG46-bearing *uvrB* excision-repair-deficient mutants TA100, TA1530, TA1535 and TA1950. STZ does not appear to require enzymatic conversion into mutagenic metabolites, since it was shown to be mutagenic for *S. typhimurium* in the absence of a liver microsomal activation system [107,109]. Zimmer and Bhuyan [110] compared the ability of several nitrosoureas to induce mutation (to histidine independence) in the histidine-requiring auxotroph *S. typhimurium* his G46 and found that STZ was almost equally mutagenic to the other nitrosoureas tested, causing 150 mutants/106 survivors at 20% lethal dose (ID₂₀) although, on a weight basis, STZ was found to be the most mutagenic of all the compounds tested. The alkylating agents STZ and *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (MNNG) were found to induce fewer mutations in TA1530 and TA1950 *uvrB* excision-repair-deficient strains than in the hisG46 excision-proficient strain, indicating that with these mutagens excision-repair is also a mutation-prone process [111]. The potent mutagenicity of STZ to strain G46 of *S. typhimurium* and its much weaker activity in strain TA1535 were also demonstrated by Liegibel et al. [86]. The relative mutagenic activities of chloroethyl-nitrosourea and methylnitrosourea antitumor agents using the Ames assay was analyzed by Franza et al. [112] who found

that all the drugs induced base substitutions. STZ was the most mutagenic of all compounds tested and showed at least a 250-fold increase in activity when compared to that of its chloroethyl analog, chlorozotocin. Besides, STZ and other nitrosoureas demonstrated an increase in mutagenicity after incubation with induced Sprague–Dawley rat liver microsomes.

STZ was also found to be mutagenic in *E. coli*, causing premutagenic lesions in mutants deficient in SOS repair or exhibiting aberrant mismatch repair [113]. Moreover, it was found that STZ induces SOS repair in *E. coli* [114]. On the other hand, Lengeler [115] characterized two classes of mutants of *E. coli* K12, resistant to STZ: (1) mutants, resistant to the highest doses of the antibiotic; (2) conditional resistant mutants which are unable to energize or to synthesize the STZ transport system (enzyme IINag-complex of the phosphoenolpyruvate (PEP)-dependent phosphotransferase system, responsible for the uptake of *N*-acetyl-glucosamine, of D-glucosamine and of STZ) under certain growth conditions but do have the transport activity under other conditions.

The sequence specificity of STZ-induced mutations in the phage *P22 mnt* repressor gene was investigated by Mack et al. [116]. Cells carrying the plasmid-borne *mnt* gene were exposed to STZ to give 10–20% survival and at least an 11-fold increase in mutation frequency. DNA sequence analysis showed that 50 of 51 STZ-induced mutations were GC to AT transitions, and one was an AT to GC transition. They also compared the STZ mutational spectrum to that for MNNG. There are sites in the *mnt* gene which are mutated only by STZ; only by MNNG, or by both agents. Sites at which only STZ induced GC to AT transition mutations occur were in sequences that are pyrimidine rich 5' to the mutated site and purine rich 3' to the mutated site.

4.2. Mammalian cells

The mutagenic activity of STZ in mammalian systems was first demonstrated in rats (1 or 10 mg/kg bw) and in mice (400 µg per mouse) [117]. A few years later, Bhuyan et al. [118] showed that STZ produces mutations to 8-azaguanine (8-AzG) resistance in V79 Chinese hamster cells. More recently, Schmezer et al. [51] using the LacI transgenic mouse mutation assay, showed that STZ induces mutations in the

DNA from liver and kidney of male C57BL/6 mice. Studies reporting mutagenesis by STZ at the HPRT locus (6-thioguanine resistant mutations, HPRT⁻) in Chinese hamster cells were made by Capucci et al. [16,77]. These authors found that the frequencies of mutants in CHO-9 and V79 cells increased as a function of STZ dose and that the rate of increase was similar for both cell lines [16]. A similar study performed in parental CHO-9 cells and its mutant hypersensitive to alkylating agents designed EM-C11, showed that the mutants were more sensitive to the mutagenic effects of STZ than the parental cell line [77]. However, none of these studies included an analysis of STZ-induced HPRT mutants in order to determine whether the induced mutations were point mutations or large deletions.

4.3. Other eukaryotes

To our knowledge, there are only two reports indicating the mutagenic effects of STZ on other eukaryotes. One is that of Browning [119], who showed that this antibiotic causes X-linked recessive lethal mutations in *Drosophila melanogaster*. More recently, Rodríguez-Arnaiz and Aranda [120] found that STZ tests positive in the w/w + somatic recombination assay in *D. melanogaster*.

5. Carcinogenesis

STZ was found to be carcinogenic in rats, mice and hamster. A single intravenous administration induces tumors in rat kidney [121], liver, and pancreas [122–125] and liver tumors in hamster [126]. Intraperitoneal administration of STZ was found to induce lung, kidney, and uterine tumors in mice; kidney, pancreatic, and liver tumors in rats [127], and hepatomas in Chinese hamsters [128]. Moreover, a recent investigation by Robbiano et al. [129] using several *N*-nitroso compounds known to induce kidney tumors in rats, showed that STZ (1 mM) induces neoplastic transformation in primary cultures of human and rat kidney cells. In addition, Shepherd et al. [130] demonstrated that STZ is a potent carcinogen in vitro for cultured rat pancreatic duct epithelial cells.

The tumorigenic capacity of STZ has been shown to be potentiated by a number of drugs including

cyclosporin A (CyA) [131], sodium barbital [132], nicotinamide [123,124,133], and poly(ADP-ribose) synthetase inhibitors [134]. As shown by Reddi et al. [131], rats rendered diabetic with a single dose of STZ followed by chronic treatment with CyA exhibit increased numbers of renal tumors, when compared to STZ treatment alone, which may reflect the nephrotoxicity of CyA and/or its immunosuppressive effects. Sodium barbital has also been shown to cause chronic nephrotoxicity by itself and to promote renal tumors at nephrotoxic doses in STZ-treated rats [132]. Moreover, there are conflicting reports of either enhanced or reduced tumor formation when animals are cotreated with STZ and nicotinamide [123,124,127,133]. In combination with poly(ADP-ribose) synthetase inhibitors, STZ promotes the development of islet β -cell tumors in rat pancreas [134].

To our knowledge, data on the carcinogenic effects of STZ in humans are still unavailable. None of the existing reports published regarding the clinical use of STZ alone or in combination with other antineoplastic agents indicates secondary drug-induced tumorigenesis [19–23], for earlier references see ref. [24]. As the International Agency for Research on Cancer (IARC) emphasizes, *STZ should be regarded for practical purposes as it were carcinogenic to humans* [135]. Accordingly, STZ is classified by the IARC within Group 2B (*The agent(mixture) is possibly carcinogenic to humans. The exposure circumstance entails exposures that are possibly carcinogenic to humans*) [136].

6. Conclusions

Current knowledge about the genotoxic effects of STZ indicates that this compound is highly mutagenic, causing DNA and chromosome damage by mechanisms which involve free radicals generated during STZ metabolism. DNA lesions produced by STZ includes monoadducts, double and single-strand breaks, and alkali-labile sites. Severe DNA damage by STZ results in cell death by apoptosis or necrosis. At the chromosome level, STZ induces micronuclei, CAs and SCEs. Despite the involvement of free radicals in the production of DNA and CAs by STZ, recent evidence suggests that these active oxygen species are not involved in the induction of SCEs by this compound. Very likely, STZ-induced SCEs result

from the alkylating action of the antibiotic on the DNA molecule. Furthermore, although both O^6 -MeG and N^7 -MeG adducts can be converted to CAs and SCEs, since more than 70% of DNA methylation by STZ occurs at the N^7 position of guanine, N^7 -MeG seems to be the predominant clastogenic and recombinogenic lesion for this compound.

Although CAs are produced by S-dependent mechanisms by alkylating compounds, present evidence shows that STZ, unlike agents with a true S-dependent action, induces both chromosome- and chromatid-type aberrations. However, like S-dependent agents, STZ is an efficient inducer of SCEs. Therefore, STZ acts on chromosomes both in an S-dependent and S-independent manner.

From a cytogenetical point of view, it is clear that more studies are needed regarding the clastogenic effects of STZ on mammalian cells. In particular, studies employing the FISH technique and combining the use of different types of DNA probes will provide more detailed information regarding the type, frequency and persistence of chromosomal aberrations induced by this antibiotic. Future areas of research also include studies about the effects of chemical mutagens (for instance, inhibitors of DNA synthesis and repair, other alkylating compounds, etc.) on the clastogenic action of STZ and studies to determine the effects of this antibiotic on the DNA and chromosomes of germ cells.

The resistance of mammalian cells to STZ is another point which merits further investigation. Although this resistance has been usually ascribed to the activity of the DNA repair protein MGMT, the existence of human and rodent cells that do not express MGMT activity and are resistant to methylation suggests that the cellular resistance of these cells to STZ may be determined by processes other than MGMT activity. Although some hypothesis have been proposed, the exact nature of these processes remains to be determined.

The scarcity of data regarding the mutagenic effects of this compound on eukaryotic cells indicates that a more intensive work on this subject is needed. In effect, little is known about the mutational spectra of STZ and the nature of the mutations induced in mammalian and other eukaryotic cells by this antibiotic.

Although there is sufficient evidence of a carcinogenic effect of this compound in several experimental animal species and the chemotherapeutic use of STZ indicates the existence of an exposed group, data on

the carcinogenic effects of STZ in humans are not available. Although STZ was found to induce neoplastic transformation in primary cultures of human and rat kidney cells [129], further studies are needed to determine whether STZ is carcinogenic for humans.

At present, the clinical use of STZ is very limited due to the development of resistance of human tumor cells and the severe toxicity and myelosuppression induced by the antibiotic. A more intensive work needs to be done regarding the mechanisms that confer resistance to STZ and the factors that can reduce the toxic effects of STZ on human subjects for this drug to become an effective antineoplastic agent. However, our recent finding that this compound is highly clastogenic and cytotoxic for colon cancer cells *in vitro* [85] suggests that a STZ-based chemotherapy for colorectal carcinomas should be reconsidered. Furthermore, since the antitumor activity of STZ was shown to be enhanced when used in combination with other chemotherapeutic drugs than when used alone, it would be very important to test the effectiveness of STZ in combination with drugs other than those previously used (vincristine, 5-fluorouracil, methyl-CCNU, procarbazine and 6-thioguanine) for the treatment of gastrointestinal cancers. The recent observation that a combination of STZ with doxorubicin produced a temporary remission of a patient's multiple pulmonary chemodectomas [25] opens a new horizon in the use of STZ as antineoplastic agent.

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