CHLOROZOTOCIN

1. Chemical and Physical Data

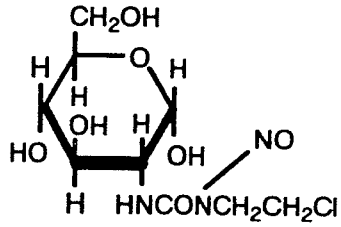
1.1 Synonyms

Chem. Abstr. Services Reg. No.: 54749-90-5

Chem. Abstr. Name: D-Glucose, 2-({[(2-chloroethyl)nitrosoamino]carbonyl}-amino)-2-deoxy-

Synonyms: D-Glucopyranose, 2-({[(2-chloroethyl)nitrosoamino]carbonyl}-amino)-2-deoxy-1-(2-chloroethyl)-1-nitroso-3-(D-glucos-2-yl)urea; 2-[3-(chloroethyl)-3-nitrosoureido]-2-deoxy-D-glucopyranose; DCNU; NSC-178248

1.2 Structural and molecular formulae and molecular weight



$C_9H_{16}ClN_3O_7$ Mol. wt: 313.69

1.3 Chemical and physical properties of the pure substance

From Windholz (1983), unless otherwise specified

(*a*) *Description*: Ivory crystals

(*b*) *Melting-point*: 147-148°C (decomposes); 140-141°C (decomposes)

(*c*) *Solubility*: Soluble in water; decomposition in aqueous solution has been studied (Montgomery *et al.*, 1975).

(*d*) *Spectroscopy data*: Infra-red and nuclear magnetic resonance spectra have been reported (Johnston *et al.*, 1975).

(*e*) *Stability*: Stable (<5% decomposition by ultraviolet spectroscopy) in solution at room temperature (22-25°C) for 3 h and at 2-8°C for 24 h; powder is stable for 24 months under refrigeration

(*f*) *Partition coefficient*: Pc = 3 (octanol:water) (Johnston *et al.*, 1975)

1.4 Technical products and impurities

Trade name: Dome

Chlorozotocin is available as a lyophilized powder in vials containing 50 mg of the compound with 48 mg citric acid and sodium hydroxide to adjust the pH (National Cancer Institute, 1988).

2. Production, Occurrence, Use and Analysis

2.1 Production and occurrence

Chlorozotocin is synthesized by nitrosation of the urea derivative prepared from D-glucosamine and 2-chloroethylisocyanate (Johnston *et al.*, 1975). It is reported to be produced in the USA.

Chlorozotocin is not known to occur naturally.

2.2 Use

Chlorozotocin is a cytostatic agent. It can be used in the treatment of cancers of the stomach, large bowel, pancreas and lung, melanoma and multiple myeloma. It has been given intravenously, at doses of 100-225 mg/m^2 (Samson *et al.*, 1982; Smith *et al.*, 1982; Bukowski *et al.*, 1983; Haas *et al.*, 1983; Forman *et al.*, 1984; Gastrointestinal Tumor Study Group, 1985). No indication for its use was given by Reynolds (1989).

2.3 Analysis

A colorimetric method for the analysis of chlorozotocin in plasma has been reported (Hoth *et al.*, 1978; Kovach *et al.*, 1979).

3. Biological Data Relevant to the Evaluation of Carcinogenic Risk to Humans

3.1 Carcinogenicity studies in animals

(*a*) *Intraperitoneal administration*

Rat: Groups of 20 male and 20 female Sprague-Dawley rats, 100 days old, were given intraperitoneal injections of chlorozotocin (synthesized according to

standard methods) at 0.4 or 2.0 mg/kg bw once a week for up to 800 days. Control groups of 20 rats of each sex received injections of Cremophor EL: ethanol:saline in a ratio of 1:1:2 volume parts. The median survival times in days were as follows: control males, 724; low-dose males, 463; high-dose males, 307; control females, 750; low-dose females, 694; high-dose females, 346. Sarcomas and mesotheliomas of the peritoneal cavity occurred in 13/20 [$p < 0.001$] and 14/20 [$p < 0.001$, Fisher's exact test] high- and low-dose males, respectively, compared to 0/20 controls, and in 16/20 [$p < 0.001$] and 10/20 [$p = 0.002$, Fisher's exact test] high- and low-dose females, respectively, compared to 1/20 controls (Habs *et al.*, 1979).

(*b*) *Intravenous administration*

Rat: Groups of 30 male Wistar rats [age unspecified] were given intravenous injections of chlorozotocin at 9.5, 19 or 38 mg/m^2 every six weeks for 10 applications. A group of 120 controls received Cremophor EL:ethanol:water in a ratio of 1.5:1.5:20 volume parts. The median survival times in days were as follows: high dose, 474; median dose, 590; low dose, 583; controls, 621. Animals were observed for life. Malignant tumours of the nervous system, lung and forestomach were found in 4, 5 and 4% of treated animals compared to 1, 0 and 1% of controls, respectively (Eisenbrand & Habs, 1980; Eisenbrand *et al.*, 1981; Zeller *et al.*, 1982). [The Working Group noted the poor survival and limited reporting.]

3.2 Other relevant data

(*a*) *Experimental systems*

The toxicity of chlorozotocin has been reviewed (Schein *et al.*, 1976; Macdonald *et al.*, 1980; Wang *et al.*, 1981; Eisenbrand, 1984; Eisenbrand *et al.*, 1986; Johnston & Montgomery, 1986).

(i) *Absorption, distribution, excretion and metabolism*

No data were available to the Working Group.

(ii) *Toxic effects*

The LD_{50} of chlorozotocin within 60 days in Sprague-Dawley rats was 27.2 mg/kg bw after intraperitoneal injection and 22.5 mg/kg bw after intravenous injection (Fiebig *et al.*, 1980).

In one study of acute toxicity, the LD_{50} after intravenous injection in BDF_1 mice was 24.9 mg/kg bw for males and 30.3 mg/kg bw for females. In animals of each sex, tubular necrosis of the kidney and cast formation were observed, as well as splenic lymphoid atrophy. In the same study, a dose of 6 mg/kg bw was lethal to beagle dogs after five days, and 3 mg/kg bw after 19 days. Renal dysfunction with tubular necrosis also occurred in these animals. Elevated serum levels of alanine aminotransferase and alkaline phosphatase were observed in dogs injected

repeatedly with chlorozotocin. Nephrotoxicity was also seen in rhesus monkeys given 40 mg/kg intravenously (Gralla *et al.*, 1979).

In Fischer rats, a lethal subcutaneous injection of chlorozotocin at 40 mg/kg bw caused renal necrosis in the cortex and, subsequently, necrosis of papillary collecting ducts. At sublethal doses, hypertrophy and karyomegaly were observed in collecting duct cells (Kramer *et al.*, 1986). In Fischer rats given a subcutaneous injection of chlorozotocin at 25 or 40 mg/kg bw, no necrosis was observed in papillary collecting ducts, although karyomegaly was observed (Dees & Kramer, 1986).

Central nervous system vascular necrosis was observed in beagle dogs treated with chlorozotocin at 1.5-2.0 mg/kg bw once a week for two weeks or with a single intraventricular dose of 10 mg/kg bw (Levin *et al.*, 1985).

Chlorozotocin affects cell cycle progression in Chinese hamster CHO cells. Non-cycling G1-arrested cells were the most sensitive; traverse from G1 to S was not affected, and chlorozotocin doubled the time for completion of DNA synthesis. Small quantities of polyploid cells were produced (Tobey *et al.*, 1975). Chlorozotocin at a concentration of 200 μM induced differentiation and inhibited cell growth of mouse neuroblastoma N-18 cells (Yoda *et al.*, 1982). DNA synthesis in L1210 leukaemia cells was almost completely inhibited (96%) within 24 h of an intraperitoneal administration to $BD2F_1$ mice (Anderson *et al.*, 1975). *In vitro*, DNA synthesis in L1210 leukaemia cells was inhibited by 68% (Fox *et al.*, 1977).

Single intraperitoneal injections of chlorozotocin at 15 mg/kg bw (maximal nonlethal dose) to BDF_1 mice slightly decreased peripheral white blood cell counts (Schein *et al.*, 1976). Similar observations were made in $CD2F_1$ mice (Fox *et al.*, 1977; Macdonald *et al.*, 1980). Intraperitoneal injections of chlorozotocin at 20 mg/kg bw to mice reduced peripheral lymphocyte counts by 50% in three days. Spleen weights were decreased by about 40%, and the response to mitogens was markedly reduced (Fisher *et al.*, 1980).

In another study in mice, chlorozotocin was shown to have immunomodulating activity. The IgM plaque-forming cell response was suppressed when the drug was injected four days before immunization; furthermore, hypersensitivity to oxazolone treatment was increased by about 30% when animals were injected intraperitoneally with chlorozotocin four days before sensitization. Treatment with chlorozotocin *in vivo* inhibited the proliferative response of spleen cells to mitogens and stimulated the chemiluminescence of peritoneal macrophages (Florentin *et al.*, 1983).

Chlorozotocin exerts its toxic and other adverse effects through the formation of mono- and bifunctional alkylating agents. It also carbamoylates proteins *via* an isocyanate intermediate formed upon decomposition (Eisenbrand, 1984).

Alkylation of nuclear chromatin in HeLa cells has been observed, and there was preferential alkylation of DNA associated with the nucleosome core particle (Tew *et al.*, 1978).

(iii) *Effects on reproduction and prenatal toxicity*

No data were available to the Working Group.

(iv) *Genetic and related effects*

Chlorozotocin induced base-pair substitutions but not frameshift mutations in *Salmonella typhimurium* in the presence and absence of an exogenous metabolic system (Zimmer & Bhuyan, 1976; Franza *et al.*, 1980; Suling *et al.*, 1983). It induced mitotic gene conversion in *Saccharomyces cerevisiae* (Siebert & Eisenbrand, 1977) and sex-linked recessive mutations in *Drosophila melanogaster* (Kortselius, 1978).

Chlorozotocin alkylated DNA in mouse leukaemia L1210 cells (Panasci *et al.*, 1979; Ahlgren *et al.*, 1982). It induced DNA strand breaks in L1210 cells (Ewig & Kohn, 1977; Alexander *et al.*, 1986) and in V79 Chinese hamster cells (Erickson *et al.*, 1978), and interstrand cross-links in DNA of mouse leukaemia L1210 cells (Ewig & Kohn, 1977) and of human embryo cells (Erickson *et al.*, 1980). It induced mutation at the *hprt* locus in V79 Chinese hamster cells (Bradley *et al.*, 1980) and sister chromatid exchange in mouse leukaemia L1210 cells (Siddiqui *et al.*, 1988) and in 9L rat brain tumour cells (Tofilon *et al.*, 1983).

Chlorozotocin at a single intraperitoneal dose of 100 μmol/kg induced DNA strand breaks and interstrand cross-links in bone-marrow cells of Wistar rats treated *in vivo* (Bedford & Eisenbrand, 1984).

(*b*) *Humans*

(i) *Pharmacokinetics*

After an intravenous dose of chlorozotocin at 120 mg/m^2, the disappearance curve of the *N*-nitroso group from the circulation exhibited three successive exponential phases, with half-times of 3-4.5 min, 6-12 min and 18-30 min. Twenty-four hours after administration of either ethyl- or glucose-labelled chlorozotocin, 82-84% of the blood-borne radioactivity was bound to protein; after seven days, 2% of the peak radioactivity value was detected in the blood. By 48 h, 50% of the radioactivity from [ethyl-^{14}C]chlorozotocin and 58% of that from [glucose-^{14}C]chlorozotocin was excreted in the urine; only 5-8% was excreted as the intact drug (Hoth *et al.*, 1978).

(ii) *Adverse effects*

Thrombocytopenia, leukopenia, elevated aminotransferase activity, nausea and vomiting were seen in patients after intravenous administration of chlorozotocin, generally at doses of 120 mg/m^2 or higher (Hoth *et al.*, 1978;

Bukowski *et al.*, 1983; Haas *et al.*, 1983; Forman *et al.*, 1984; Schutt *et al.*, 1984; Gastrointestinal Tumor Study Group, 1985).

(iii) *Effects on reproduction and prenatal toxicity*

No data were available to the Working Group.

(iv) *Genetic and related effects*

No data were available to the Working Group.

3.3 Case reports and epidemiological studies of carcinogenicity to humans

No data were available to the Working Group.

4. Summary of Data Reported and Evaluation

4.1 Exposure data

Chlorozotocin has been used as a cytostatic drug for the treatment of cancers at a variety of sites.

4.2 Experimental carcinogenicity data

Chlorozotocin was tested for carcinogenicity in single experiments in rats by intraperitoneal and intravenous injection. Intraperitoneal administration induced a high incidence of sarcomas and mesotheliomas in the peritoneal cavity in rats of each sex. The study by intravenous administration was inadequate for evaluation.

4.3 Human carcinogenicity data

No data were available to the Working Group.

4.4 Other relevant data

Chlorozotocin alkylates DNA and protein and causes DNA interstrand cross-links. In humans, it induces leukopenia and thrombocytopenia; in animals, it suppresses the bone marrow and affects immune response.

It is hepatotoxic in both humans and experimental animals.

Chlorozotocin induced DNA damage in bone-marrow cells of rats *in vivo*. It induced DNA damage in human, mouse and Chinese hamster cells *in vitro*, sister chromatid exchange in mouse and rat cells and gene mutation in Chinese hamster cells. It induced sex-linked recessive lethal mutations in *Drosophila* and gene

conversion in *Saccharomyces cerevisiae*. Chlorozotocin induced mutations in *Salmonella typhimurium*. (See Appendix 1.)

4.5 Evaluation[1]

There is *sufficient evidence* for the carcinogenicity of chlorozotocin in experimental animals.

No data were available from studies in humans on the carcinogenicity of chlorozotocin.

In making the overall evaluation, the Working Group also took note of the following information. Chlorozotocin is an alkylating agent and is structurally related to other chloroethyl nitrosoureas, one of which, 1-(2-chloroethyl)-3-(4-methylcyclohexyl)-1-nitrosourea (methyl-CCNU), is carcinogenic to humans (Group 1) and two of which, bischloroethyl nitrosourea (BCNU) and 1-(2-chloroethyl)-3-cyclohexyl-1-nitrosourea (CCNU), are probably carcinogenic to humans (Group 2A) (IARC, 1987). Chlorozotocin has given consistently positive results in a broad spectrum of assays for genetic and related effects, including those involving mammalian cells.

Overall evaluation

Chlorozotocin *is probably carcinogenic to humans (Group 2A).*

5. References

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[1]For description of the italicized terms, see Preamble, pp. 26–29.

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