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1,3-*bis*-Chloroethyl-1-Nitrosourea (BCNU)

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Paper IV

Abbreviations:
BCNU: 1,3-*bis*-Chloroethyl-1- nitrosourea
CENU: (chloroethyl)nitrosourea
CCNU: 1-(2-Chloroethyl)-3-cyclohexyl-1-nitrosourea
GR: Glutathione Reductase
GSH: Glutathione
GSSG: Glutathione Disulfide
MNU: 1-Methyl-1-nitrosourea

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Abstract

1,3-*bis*-Chloroethyl-1-nitrosourea (BCNU) is an antineoplastic drug widely used for the treatment of numerous tumors. The stability of BCNU is pH-dependent and temperature-dependent. The half-life of BCNU also varies in different buffers. BCNU is unstable in aqueous and decomposes chemically under physiological conditions to yield alkylating and carbamoylating intermediates. The alkylating moiety, chloroethyl carbonium ion intermediate, has the antitumor activity by forming DNA monoadducts and cross-linking DNA. The carbamoylating moiety, isocyanate, reacts with proteins and causes enzyme inactivation. Glutathione reductase(GR), an enzyme involved in oxidative pathway, loses activity when carbamoylated by BCNU. This paper will focus on the structure, stability, chemistry, and the biological effects of decomposition and the clinical usage of BCNU.

Introduction

The CENU, a nitrosourea family, is an important class of clinically useful antitumor agents. Clinically useful members of this class are BCNU and 1-(2-chloroethyl)-3-cyclohexyl-1-nitrosourea (CCNU). BCNU, also called carmustine, is a chemotherapy agent that has been been used since 1971 in the treatment of multiple myeloma, malignant melanoma, breast cancer, gastrointestinal cancers, Burkitt's lymphoma and primary or metastatic brain tumors. It has also been reported to have antiviral, antibacterial, and antifungal activity. It is unstable under physiological conditions, and undergoes decomposition reactions in aqueous solution, generating reactive intermediates known to modify both DNA and protein [1].

Properties and Structure of BCNU:

The structure of BCNU is shown in figure 1. It is a two-haloethyl component of 1-methyl-1-nitrosourea molecule (MNU). MNU has the ability to cross the blood-brain barrier at sufficiently high level and lead to produce more active nitrosoureas. N- (2-haloethyl)-N – nitrosoureas, one specific structural type, has been proved superior to MNU with respect to activity on a weight basis. At this point, the 2-haloethyl and the N-nitroso structure are considered necessary for the antitumor function of BCNU [1,7].

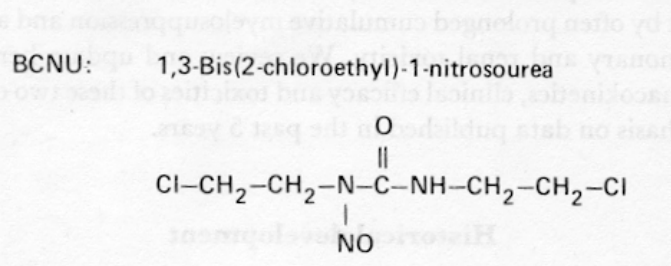

Figure 1: **The structure of BCNU**. From [2].

BCNU is an orange-yellow solid and molecular weight is 214.06 Da. This compound is slightly soluble in water (<1 mg/mL at 18°C), soluble in DMSO, ethanol or acetone (>100 mg/mL at 18°C), and highly soluble in lipids. Its low molecular weight and high lipid solubility allows BCNU to cross the blood-brain barrier easily. It is not only sensitive to oxidation but also

hydrolysis, subsequently forming alkylating and carbamoylating intermediates. It can decompose rapidly in acid and in solutions at pH > 7, but is most stable in petroleum ether or aqueous solution at pH 4. This chemical is stable under normal laboratory conditions and will decompose at a temperature of 30.5ºC. When heated to decomposition, it emits toxic fumes of hydrochloric acid and other chlorinated compounds as well as nitrogen oxides. Therefore, it must be stored in a refrigerator before use [3].

Stability of BCNU

The aqueous decomposition of BCNU is relatively rapid and is pH-dependent. At pH 6.0 in buffer, BCNU has half-life of 314 minutes; while at pH 7.4, the half- life is 52 minutes [4]. In serum, the rate of decomposition of BCNU is significantly higher than in aqueous buffer at the same pH and temperature. This high rate of breakdown is due to the interaction of serum proteins and BCNU, in plasma at pH 7.4, the half- life of BCNU is 17 minutes [4].
BCNU stability also varies at different buffer concentration and temperature. The result of many studies suggest that, although the decomposition rates of BCNU are significantly increased at body temperature (37ºC), it is quite low and essentially unaffected by the different dilution at room temperature (19.5ºC) [5].

Because of the stability problem, this compound has been studied for many method of storage. It has demonstrated that when stored at 4ºC in the dark, BCNU admixture can be prepared up to 48 hours before administration, either in 5% dextrose or in 0.9% sodium chloride isotonic solutions. However, storage should not exceed 72 hours. Either glass flasks or Stedim 6 bags can be used store admixture. Stedim 6 bags are preferable because they are lighter than flask and do not need to be closed with rubber stoppers, which may promote absorption or contaminate the solution [6].

Chemistry of BCNU

The nitrosoureas are class of highly active antitumor agents, one of them BCNU is currently in clinical use. It is believed that the cytotoxicity of the compound is due to its decomposition to

alkylating moiety and carbamoylating moiety. The general decomposition scheme for BCNU can be seen in figure 2. The alkylating moiety is further decomposed to the carbonium ion and reacts in the cell to form DNA monoadducts and cross-linked DNA. The carbamoylating moiety, isocyanate, reacts with the –SH, -OH, and –NH_2 groups of proteins and causes enzyme inactivation.

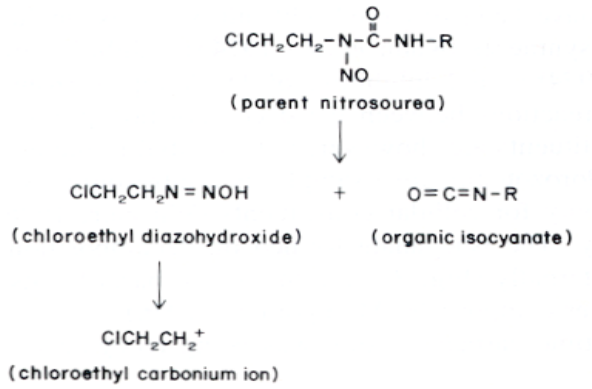

Figure 2: Abbreviated scheme for deposition of a nitrosourea [7].

At different pH conditions, there are two competing mechanisms of decomposition: at pH 7.4, the decomposition is predominantly through 2-chloroethyldiazohydroxide, and chloroethanol and acetaldehyde are the major products (Figure 3); at pH 5.0, decomposition is predominantly through 4,5-dihydro-1,2,3-oxadizole and 2-hydroxyethyldiazohydroxide, and ethylene glycol and acetaldehyde are the major products [8].

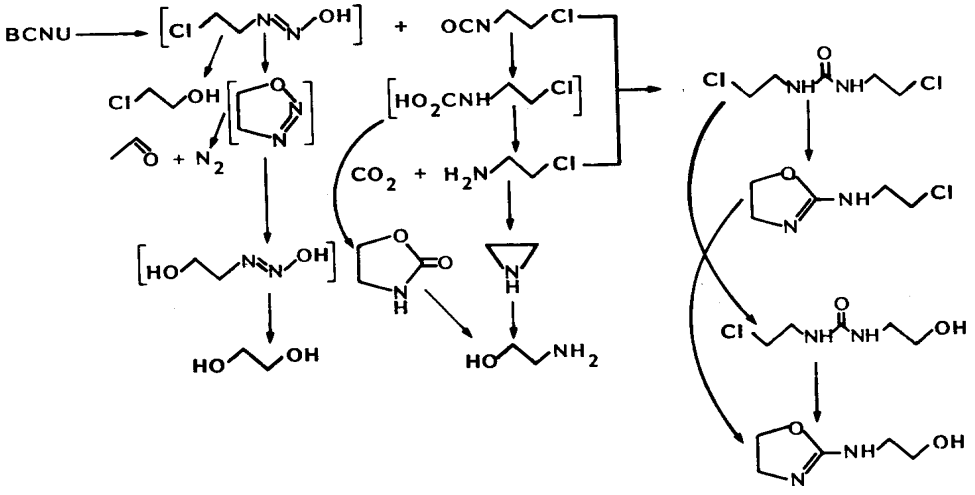

Figure 3: Aqueous decomposition of BCNU at pH 7.4 (37ºC) [9]. The various products are concentration dependent

Reaction of BCNU

BCNU has both alkylating and carbamoylating activities. The former reactions are attributed to nucleic acid alkylation that is the principal basis for antitumor activity of nitrosoureas, toxic side effects; the latter are attributed to protein carbamoylation.

Carbamoylation

It is quite clear that carbomoylation is the basis for many of the biochemical actions, including inhibition of macromolecular syntheses, inhibition of DNA repair. The carbamoylation reaction occurring between an isocyanate formed during decomposition of BCNU and a reactant capable of losing a proton involves formation of a covalent bond between the isocyanate and its reactant (Figure 2) [10]. Isocyanates are highly reactive compounds and the reaction is spontaneous and rapid. The number and range of possible site for carbamoylation in biologic system are vast.

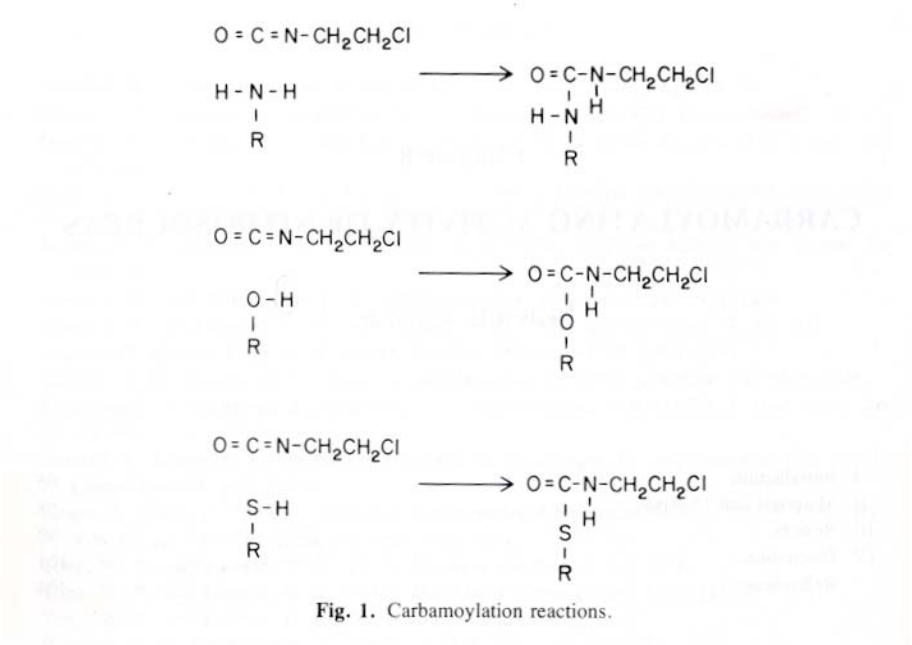

Fig. 1. Carbamoylation reactions.

Figure 4: Carbamoylation reactions[10].

Reaction with DNA:

Tumor cell DNA is the primary target of many effective anticancer agents. Thus, DNA alkylating agents occupy a key position in the currently available chemotherapeutic arsenal. As

an alkylating agent, BCNU causes DNA damage by modifying bases, cross-linking, and inducing DNA strand breaks [11].

1) Base Modification

BCNU alkylates DNA predominantly (>90%) at *N*7 positions of guanine and to a less extent, at the *O*6 position of guanine [1]. Although *N*7 guanine adducts can exist in cells for a long time without lethal effect , they are prone to imidazole ring rupture . Decomposition of these adducts through the rupture of the imidazole ring will give rise to an altered base, a formamidopyrimidine, which can block DNA synthesis [12] and are lethal if not repaired.

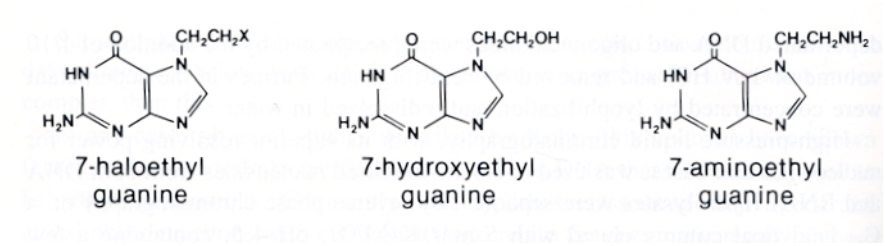

Figure 5: Base modified by BCNU [13].

2) cross-linking of DNA

The cross-linking of DNA is believed to be the primary event responsible for the anticancer activity of most of the clinically useful alkylating agents.

It is proposed that guanine-O_6 position is sensitive to chloroethylation [14]. It is found the cross-links to be uniformly distributed throughout the GC- rich region, which suggests that one source of BCNU interstrand cross-links is linkage of deoxyguanosine and deoxycytidine partners. It is generally accepted that the DNA-DNA interstrand cross-links formed in duplex DNA by BCNU bridge N1 of G and N3 of C residues. The drug's active metabolite is the chloroethyl carbonium intermediate, which reacts with the O^6 position of guanine. This is followed by an intermolecular rearrangement forming an intramolecular N^1,O^6-ethanoguanine adduct. Ultimately, the exocyclic C-O bond is cleaved, and reaction with the N^3 position of cytosine in the complementary strand results in the N^3C-N^1G diadduct, which appears to be the

cytotoxic molecular lesion [15]. The presently accepted mechanism of DNA interastrand cross-linking by BCNU is showed as figure 6.

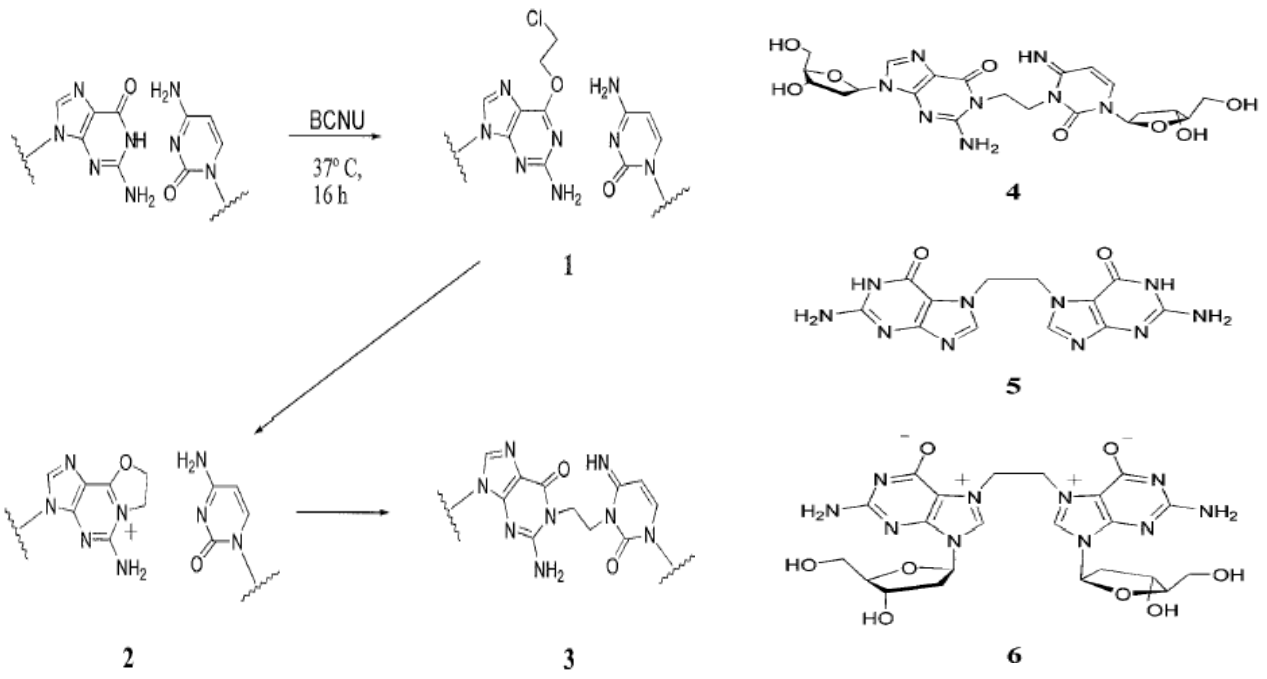

Figure 6: Presently accepted mechanism of DNA interastrand cross-linking by BCNU [15].

Reaction with GR

Extensive carbamoylation reaction is responsible for the inactivation of proteins, including glutathione reductase (GR), thioredoxin reductase, alcohol dehydrogenase, transglutaminase. GR, acting as an important antioxidant, reduces glutathione disulfide (GSSG) back to primary co-factor glutathione (GSH). BCNU can modify GR at Cys 58 and be inactivated rapidly [16]. The figure 7 shows the scheme of these reactions.

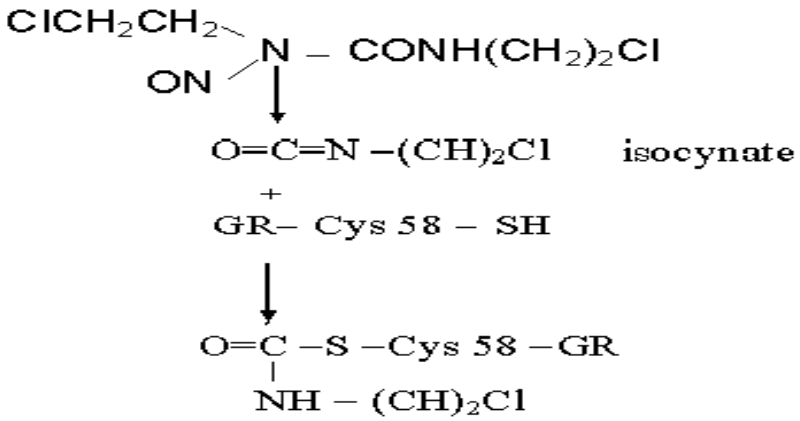

Figure 7: Inhibition GR activity by BCNU. Adapt from [16].

Clinical Activity

BCNU is an antineoplastic drug widely used for the treatment of solid tumors, lymphomas and in preparative regimens for bone marrow transplantation. Because of its ability of penetrating the blood-brain barrier rapidly, BCNU is used for treatment of malignant brain tumors. BCNU has side effect when it using in high dose. The major toxicities of BCNU in cell transplantation setting are to the lung, liver and kidney. The most important acute toxicity is myelosuppression. Pulmonary fibrosis and kidney insufficiency are the common chronic toxicities [17].

Summary:

BCNU with anticancer activity against human tumor is a clinically useful chemotherapy agent. It shows to be capable of alkylating and cross-linking DNA which is highly effective in model tumor systems and carbamoylating and inactivate proteins. BCNU can decrease the level of GSH in cell by inhibiting GR acitivity. This agent has been used effectively in the treatment of lymphomas and a variety of human solid tumors including brain tumors; however, its utility is limited by serious toxicities.

References:

1. Tong WP, Kirk MC, Ludlum DB. (1982) Formation of the cross-link 1-[deoxycytidyl]-2-[*N*1-deoxyguanosinyl]-ethane in DNA treated with *N*, *N9*-bis (2-chloroethyl)-*N*-nitrosourea. *Cancer Res.* **42:** 3102–3105.
2. Beverley P (1994): Physicians' Desk Reference, 48th ed. "BiCNU" Oradell: Medical Economics Co., pp651-653.
3. Raymond BW, Brian FI. (1982) The nitrosoureas: carmustine(BCNU) and lomustine(CCNU). *Cancer Treatment Rev.* **9:** 313-330.
4. Loo TL, Dion RL. (1965) Colorimetric method for the determination of 1,3-1,3-*bis*-Chloroethyl-1-nitrosourea (2-chloroethyl)-1-nitrosourea. *J Pharmaceutical Sci.* **54:**809-810.
5. Tepe P. Hassenbusch SJ. Benoit R. Anderson JH. (1991)BCNU stability as a function of ethanol concentration and temperature. *J Neuro-Oncology*. **10:**121-127.
6. Hadji-Minaglou-Gonzalvez MF. Gayte-Sorbier A. Airaudo CB. Verdier M. (1992)Effects of temperature, solution composition, and type of container on the stability and absorption of carmustine *Clinical Therapeutics*. **14:**821-824.
7. Herbert E, Kann Jr.(1981) Carbamoylating activity of nitrosoureas. In Prestayko AW. ed. *Nitrosoureas Current Status and New Developments.* New York: Academic Press. pp.95- 105.
8. Brundrett RB. (1980) Chemistry of nitrosoureas. Intermediacy of 4,5-dihydro-1,2,3-oxadiazole in 1,3-*bis*(2-chloroethyl)-1-nitrosourea decomposition. *J Med Chem.* **23:**1245-1247.
9. Montgomery JA. (1981) The chemistry of the nitrosoureas. In Serrou B. ed. *Nitrosoureas in Cancer treatment.* New York: Academic Press. pp. 13-19.
10. Herbert E, Kann Jr. (1981) Carbamoylating activity of nitrosoureas. In Prestayko AW. ed. *Nitrosoureas Current Status and New Developments.* New York: Academic Press. pp. 95-105
11. Wiencke JK, Wiemels J. **(**1995) Genotoxicity of 1,3-bis(2-chloroethyl)-1-nitrosourea (BCNU). *Mutat Res* **339:** 91–119.
12. Muller N, Eisenbrand G**.** (1985) The influence of N7 substituents on the stability of N7-alkylated gunosines. *Chem Biol Interact **55:***173–181.
13. David BL, William PT. (1981) Modification of DNA and RNA bases.In Prestayko AW. ed. *Nitrosoureas Current Status and New Developments.* New York: Academic Press. pp. 85-95.
14. Kohn KW. (1977) Interstrand cross-linking of DNA by 1, 3-bis (2-chloroethyl)-1-nitrosourea and other 1-(2-haloethyl)-1-nitrosoureas. *Cancer Res.* **37:**1450-1454.
15. Manny DB, Stewart P.(2002)Mechanisms of Resistance to 1,3-Bis(2-chloroethyl)-1-nitrosourea in Human Medulloblastoma and Rhabdomyosarcoma. *Mol Cancer Ther* **1:**727-736.
16. Frischer H. Ahmad T. (1977) Severe generalized glutathione reductase deficiency after antitumor chemotherapy with BCNU [1,3-*bis*(chloroehtyl)-1-nitrosoureal]. *J Lab Clin Med*. **89:**1080-1091.
17. Carter SK, Schabel FM, Broder LE, Johnston TP. (1972) 1,3-*bis*(2-chloroethyl)-1-nitrosourea (bcnu) and other nitrosoureas in cancer treatment: a review. *Advan Cancer Res.* **16:**273-332.