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

by

Wenqing Sun

Free Radical & Radiation Biology Program

B-180 Medical Laboratories

The University of Iowa

Iowa City, IA 52242-1181, USA

Phone: (319) 384-4454

Fax: (319) 335-8039

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Abbreviations:

BCNU	1,3-*bis*-Chloroethyl-1-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 a lipid soluble antitumor drug. It is unstable in aqueous solution and degrades spontaneously to reactive alkylating and carbamoylating intermediates. The alkylating component is believed to be responsible for the antitumor effects of this drug and the carbamoylating species mediate some of the serious adverse effects of BCNU therapy. BCNU covalently deactivates glutathione reductase (GR) by alkylcarbamoylating their thiolate active sites. The depletion of active GR leads to the decrease in the GSH/GSSG ratio, especially in hyperoxia status. Clinically, BCNU is used in the treatment of brain tumors, lymphomas, myeloma and lung cancers. However, its suppressing effect on bone marrow limits both the frequency of administration and total dose of BCNU. This paper will focus on the structure, decomposition, the biological effects of decomposition and the clinical usage of BCNU.

Introduction

1,3-*bis*-(2 Chloroethyl)-1-nitrosourea (BCNU), also called carmustine, is a member of the nitrosourea family, one of the most extensively studied classes of anticancer agents. In the early 1960s, BCNU was first found to be curative against both intraperitonial and intracerebral leukemia [1]. In aqueous solution, the chemical stability of BCNU is limited; it is rapidly decomposed to alkylating and carbamoylating agents. This paper will focus on the structure, decomposition, the biological effects of decomposition and the clinical usage of BCNU.

Structure of BCNU

The chemical structure of BCNU is shown in figure 1. It is a two-haloethyl component of 1-methyl-1-nitrosourea molecule (MNU), MNU itself shows antitumor activity.

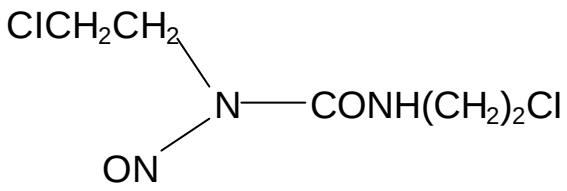

Figure 1. The structure of the BCNU. From [2]

The N-nitroso structure is necessary for the antitumor function. Other haloethyl compounds, such as 2-iodoethyl and 2-bromoethyl are inactive or less active than BCNU.

BCNU has a molecular weight of 214.06 g per mole. Its low molecular weight and high lipid solubility allows BCNU to cross the blood-brain barrier easily.

Reaction of BCNU

1) Decomposition

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 [3]. The decomposition products of BCNU form both an alkylating moiety and a carbamoylating moiety. 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. At different pH conditions, there are two competing mechanisms of decomposition [4]: 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; at pH 7.4, the decomposition is predominantly through 2-chloroethyldiazohydroxide, an chloroethanol and acetaldehyde are the major products.

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 [3].

2) Denitrosation

Although the chemical reactivity of BCNU suggests that its initial metabolic fate in the body would depend primarily upon spontaneous decomposition, BCNU can still undergo rapid denitrosation transformation to form parent urea. The denitrosation is catalyzed by liver microsomes in the presence of NADP and phenobarbital can increase this reaction. *In vivo,* denitrosation of BCNU results in decreased antitumor activity and reduction of systemic toxicity.

Reaction With Nuclear Acid

1) Base Modification

BCNU causes base modification *via* two mechanisms: the first mechanism consists of a single substitution at a nucleophilic site to form haloethyl, hydroxyethyl or aminoethyl derivatives. The second mechanism involves a two-step modification to form the ethano or diguanylethane derivatives. Among these, aminoethylguanine formation involves the reaction of the 3-position chloroethyl group in BCNU. This reaction is unique to BCNU and probably is

responsible for the mutagenic or carcinogenic effects of BCNU. Figure 2 shows the mechanism of BCNU modification bases.

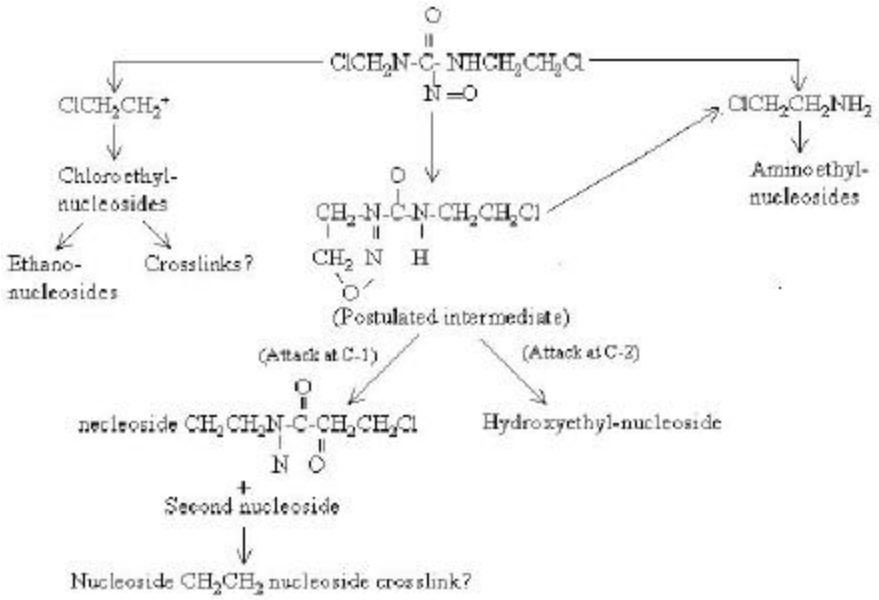

Figure 2. The scheme of BCNU modification of bases. From [5]

2) DNA Crosslink

BCNU induced DNA crosslinking arises from its alkylation reactions and contributes to the cell killing and antitumor activity of BCNU.

DNA crosslinking involves essentially two steps: first BCNU generates highly reactive intermediates that transfer chloroethyl groups to a nucleophilic site on one DNA strand, then the resulting chloroethyl monoadducts react with a second nucleophilic site via displacement of chloride to form an ethylene bridge between the two nucleophilic sites. The mechanism of DNA crosslinking is shown in Figure 3. It is proposed that guanine-O^6 position is sensitive to chloroethylation [6]. The formation of ethylene bridge is slow, requiring several hours for completion, thus providing ample time for a DNA repair process.

BCNU can also cause DNA-protein crosslinking by initiating chloroethylation at the amino or sulfhydryl group of protein [7].

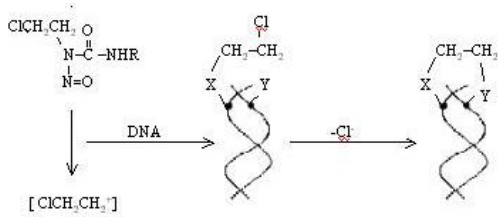

Figure 3. Scheme of the mechanism of DNA interstrand crosslinking by BCNU. X and Y are nucleophilic sites located on opposite DNA strands. From [6]

Reaction With Protein

BCNU has both alkylating and carbamoylating activities, the former reaction is attributed to nucleic acid alkylation and the latter to protein carbamoylation.

The carbamoylation reaction occurring between an isocyanate and a reactant capable of losing a proton involves formation of a covalent bond between the isocyanate and its reactant (Figure 4). Isocyanates, formed on decomposition of BCNU, are highly reactive compounds; therefore, the carbamoylation reaction is spontaneous and rapid.

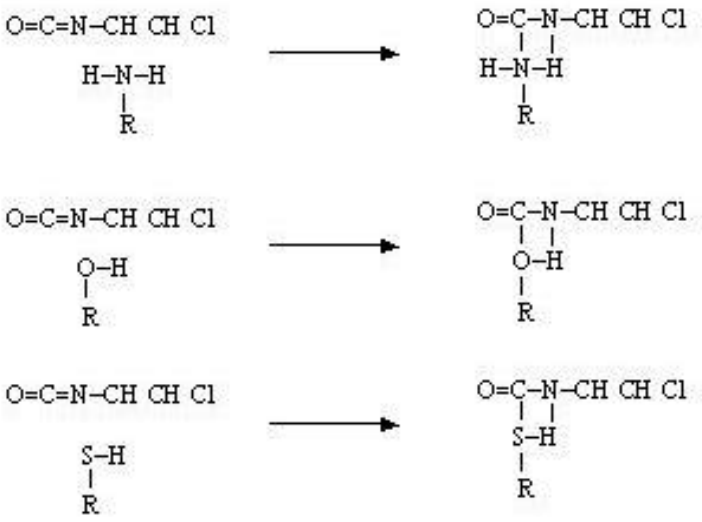

Figure 4. Carbamoylation reactions

From [8]

Extensive carbamoylation of protein by BCNU *in vivo* results in the inactivation of proteins, such as chymotrypsin, alcohol dehydrogenase and glutathione reductase [9]. Glutathione reductase (GR), a NADPH-dependent antioxidant, can be inactivated by BCNU rapidly. Cohen [10] found that incubation of leukemia cells with 10 μM BCNU caused total inhibition of GR in 7 minutes. After removing BCNU, 59% of activity recovered within 12

hours. The recovery of activity is dependent on *de novo* protein synthesis. Crystallographic analysis demonstrates that the inhibition is due to the attack on the thiol of cys-58 of GR.

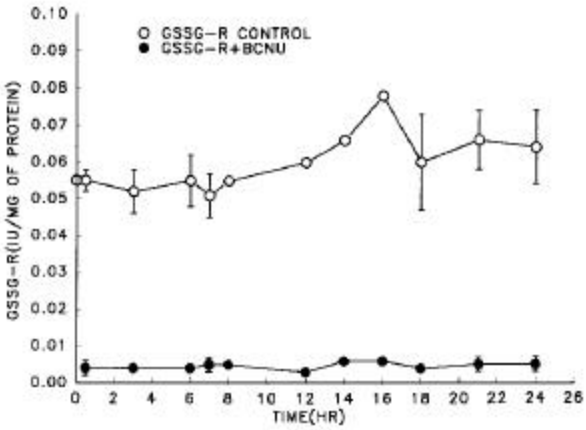

Figure 5. Glutathione reductase activity as a function of time in cells treated with BCNU. The activity of glutathione reductase was measured as the function of time in K562 cells treated with 1.25 x 10^{-5} M BCNU (closed circles) or buffer (open circles). Upon exposure to BCNU, glutathione reductase was rapidly inactivated, and this persisted throughout the experimental period. There was no loss of enzyme activity in the no-drug control cells. From [11]

The inhibited GR shows decreased affinity for NADPH and mercaptoethanol protects GR from inhibition by competing with BCNU [12].

Acting as an important antioxidant, GR catalyzes the recycling of glutathione (GSH). GSH supports many cell functions including proliferation, detoxification, enzyme protection and drug metabolism. Under oxidative stress, GSH removes H_2O_2 and becomes glutathione disulfide (GSSG). The recycling of GSSG depends exclusively on GR. Under the condition of an increased oxidizing environment, inhibition of GR can significantly increases the amount of GSSG and decreases the ratio of GSH/GSSG, thus it enhances the oxidative stress of organs [13].

Analysis of BCNU

Several techniques have been used to analyze BCNU in plasma and other biological samples. These techniques comprise spectrophotometry, gas chromatography-mass spectrometry, chemical ionization-mass spectrometry and capillary gas chromatography assay. Among these assays, capillary gas chromatography is rapid, accurate and specific; the minimum analyzable concentration is 0.003 μg/ml [14]. **Figure 6 shows the typical capillary gas chromatograms of BCNU.**

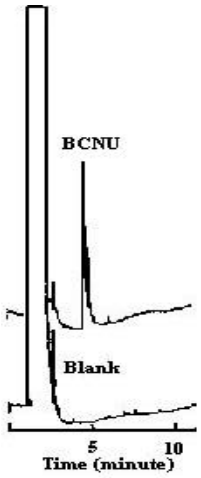

Figure 6. Typical capillary gas chromatograms of BCNU. An intact blank human plasma sample (b) and the typical chromatograms peaks generated for the Blank human plasma with un-derivative BCNU (s) were shown. The concentration for BCNU was 0.07 μg/ml. The retention time for BUCN was 4.7 minutes. BCNU was extracted from plasma with benzene; the extraction efficiency is higher than 87%. From [14].

Clinical Usage and Side-Effects

BCNU demonstrates its antitumor activity in a wide range of tumor spectrum, including cerebral tumors, myelomas, lymphomas and melanomas. Especially because it can penetrate the blood-brain barrier rapidly, it is used for treatment of malignant brain tumors.

The major organs of toxicity for BCNU include the bone marrow, lung, liver and kidney. The most important acute toxicity is myelosuppression. Myelosuppression can also be seen with other chloroethyl nitrosoureas such as CCNU and methyl CCNU [15]. Pulmonary fibrosis and kidney insufficiency are the common chronic toxicities. These adverse toxicities are dose related. Takvorian [16] recommended the maximal safe single dosage of BCNU should be equal to or less than 1200 mg/m^2.

Summary

BCNU is a chemotherapeutic drug that decomposes to form both alkylating agents that cause DNA crosslinking and carbamoylating agents that inactivates proteins. It inactivates GR activity thus decreases the ratio of GSH/GSSG and enhances the oxidative stress of organs. Clinically BCNU shows antitumor effect on a wide range of tumor spectrum. However, its dose is limited by its toxicity.

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