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Bleomycin

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Abbreviations

BLM	Bleomycin
MPO	Myeloperoxidase
NOS	Nitric oxide synthase
PMN	Polymorphonuclear
PVB	Cisplatin, vinblastine, bleomycin
RNS	Reactive nitrogen species
ROS	Reactive oxygen species
VAB	Vinblastine, actinomycin, bleomycin

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Abstract

Bleomycins (BLMs) are cytotoxic glycopeptides different from one another only in their terminal amine. BLMs have the dual properties of binging to DNA and chelating various metal irons, notably iron and copper. The bleomycin-ferrous complex can produce the superoxide radical and the very toxic hydroxyl free radical, which was shown to break the C3' to C4' bond of deoxyribose in DNA strands. The net result is both single- and double-strand breaks in DNA, with resulting chromosomal deletion and fragmentation. The reaction is enhanced by radical-producing compounds. Bleomycin-mediated DNA degradation requires the presence of a redox-active metal ion such as Fe^{2+} or Cu^{+}, as well as molecular oxygen. Through above mechanism, BLMs are active drug in chemotherapy in various cancers.

Introduction

The bleomycins (BLMs) are cytotoxic glycopeptides produced by a strain of the actinomycete *Streptomyces verticillus*. The mixture of glycopeptides that comprise the clinically used drug bleomycin was iosolated, purified, and characterized in Japan by Umezawa and co-workers [1]. Since their discovery, the bleomycins have been the focus of detailed structural, biosynthetic, synthetic, mechanistic, and therapeutic investigations. They are presently employed clinically in combination with a number of other agents for the treatment of several types of tumors, notably squamous cell carcinomas and malignant lymphomas [2]. The bleomycins are commonly employed therapeutically as a mixture of several congeners denoted blenoxane, which consists predominantly of bleomycin A_2 and bleomycin B_2. The efficacy of bleomycin as an antitumor agent has been established, for example, by the finding that omission of bleomycin from a multidrug regimen employed for the treatment of germ cell carcinomas resulted in a substantial lessening of efficacy [3].

Chemistry of Bleomycins

All BLMs are soluble in water and methanol and insoluble in other organic solvents and show two absorption maxima at 242-246 nm and 291-293 nm [4]. All BLMs are different from one another in the terminal amine moieties. Each of the bleomycin peptides has a molecular weight of ~1500. The bleomycinic acid portion of the molecule contains six nitrogens thought to participate in metal binding, notably chelating iron and copper [5]. Copper-free BLMs undergo reaction with BLM hydrolase and the β-aminoalaninamide moiety is hydrolyzed but copper-chelated BLMs are resistant to this enzyme reaction. Gas chromatographic analysis of the trimethylsilyl derivatives of methanolysis products of BLMs indicates that all BLMs contain the

same sugar moiety while hydrolysis study indicates that all natural BLMs are different from one another in their terminal amine moieties [6].

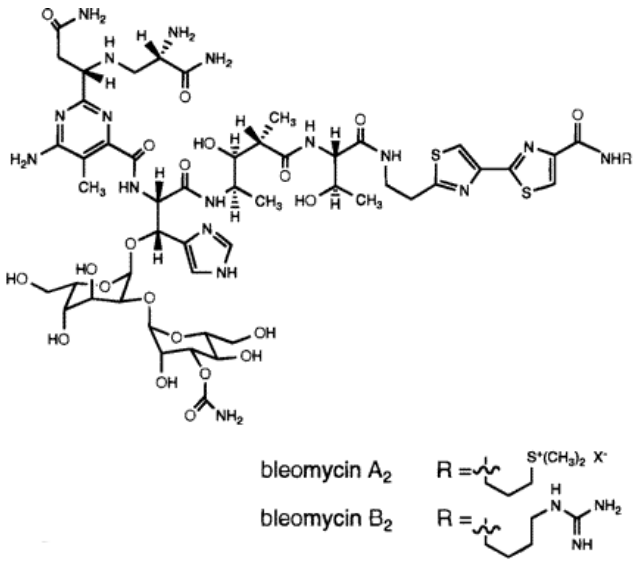

Figure 1. Structure of Bleomycin [7]

As shown in Figure 1, bleomycin contains at least four functional domains. These include the metal-binding domain, which is responsible for metal ion binding and O_2 activation and which must ultimately mediate the abstraction of H-atoms from the DNA substrate [8]. The bithiazole and C-terminal substituent are known to be involved in DNA binding. Removal of the C-terminal substituent or introduction of a substituent that lacks a positive charge under the conditions of DNA cleavage dramatically diminishes the efficiency of DNA cleavage by bleomycin [9]. The carbohydrate moiety is the least well characterized of the functional domains of bleomycin. This domain seems likely to participate in cell recognition by bleomycin and possibly in cellular uptake and metal ion coordination [8].

As regards the metal-binding domain, numerous studies have documented the ability of this portion of the BLM molecule to bind numerous metal ions. A more surprising finding is that

the metal-binding domain also constitutes the primary determinant of the sequence selectivity of DNA cleavage by BLM. Evidence in support of this conclusion includes the finding that the alteration of the C-terminal substituent of BLM had no effect on the strand selectivity of cleavage at a high-efficiency DNA cleavage site, while alteration of the metal-binding domain exhibited dramatic effects in altering strand selectivity [10]. A second line of evidence was provided by Mascharak and co-workers, who demonstrated that a preformed Fe(III) complex of a ligand structurally related to the metal-binding domain of BLM gave a DNA cleavage pattern virtually indistinguishable from that of Fe(III)·BLM itself when each was activated with H_2O_2. Presumably, the stoichiometric activation of this molecule from a preformed Fe(III) complex must compensate for the diminished DNA affinity resulting from the absence of the DNA-binding domain.

Mechanism of Action

The mechanism of action of bleomycin is unique among anticancer drugs and has been a subject of intense interest to biochemists and molecular biologists. The drug has the dual properties of binging to DNA and chelating various metal irons, notably iron and copper. The bleomycin-ferrous complex, in particular, has been shown to function as a mini enzyme, catalytically reducing oxygen and producing various free radicals [11, 12]. Grollman and Takeshita have postulated that DNA breakage occurs by cleavage of the C3’ to C4’ bond of deoxyribose, and this cleavage is mediated by the complex of bleomycin, ferrous iron, and molecular oxygen. Alternatively, this complex has been shown to produce the superoxide radical and the very toxic hydroxyl free radical, which could produce similar damage.

The net result is both single- and double-strand breaks in DNA, with resulting chromosomal deltions and fragmentation. Bleomycin is generally more active against dividing cells than nondividing cells and demonstrates specificity for the G_2 and M phase of the cell cycle. The chemistry of the DNA breakage is thought to involve abstraction of a hydrogen atom from deoxyribose at the C4' position by the free radical species and subsequent attack by an oxygen molecule to form a peroxide. The opening of the deoxyribose ring leads to liberation of free bases, a glycolic acid ester, and a base-propanol, which subsequently can be degraded to liberate malondialdehyde. Thymine is released preferentially, with some release of the other three bases as well. There appears to be some preference for the GpT sequence of DNA, and cleavage appears to occur specifically at the 3' end of guanine.

It has been proposed that the major mechanism of resistance to bleomycin is an increase in the level of a cytosolic enzyme termed "bleomycin hydrolase". This aminopeptidase inactivates the drug and is virtually absent in lung and skin, the two normal tissues most susceptible to the drug.

It has been shown that hypoxia is a mechanism of resistance to bleomycin in cultured cells, and this may be an important resistance mechanism in human solid tumor with a large hypoxic cell fraction. Since the drug also acts preferencially on cells that are dividing, especially those in the G2 and M phases of the cell cycle, tumors with a low growth fraction might be expected to be resistant to the drug on a cytokinetic basis.

Bleomycin-mediated DNA degradation requires the presence of a redox-active metal ion such as Fe^{2+} or Cu^{+}, as well as molecular oxygen [13].

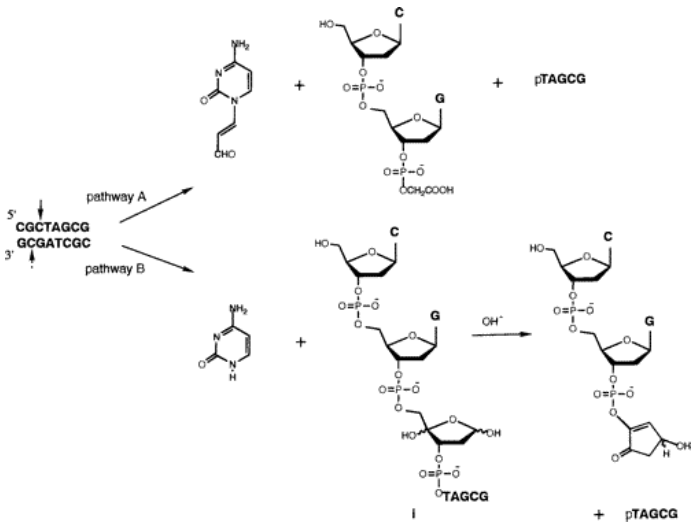

Figure 2. Products resulting from degradation of a self-complementary oligonucleotide by Bleomycin [7]

As illustrated in figure 2, using the self-complementary DNA octanucleotide $^{5'}$CGCTAGCG$^{3'}$ as a substrate, bleomycin produces two sets of products from B-form DNA substrates. One of these (pathway A) results in frank DNA strand scission [14], while the other (pathway B) affords base release at the site of the BLM-induced lesion with concomitant formation of a 4'-hydroxyapurinic acid moiety (the "alkali-labile" lesion) [15]. The latter intermediate can be induced to undergo DNA strand scission by admixture of any of a few different reagents, including alkali, alkylamines, or hydrazine. It may be noted that both sets of products derive from a common intermediate, namely, an initially formed C-4' radical resulting from the abstraction of an H-atom from the DNA substrate by bleomycin. This radical intermediate can combine with dioxygen, forming a hydroperoxy radical; fragmentation of the oxygenated sugars via a Criegee-type process then affords a base propenal and an oligonucleotide terminating in a 3'-phosphoroglycolate moiety (Figure 2). Alternatively, the sugar radical can undergo oxidation, perhaps mediated by bleomycin itself, to form a carbocation that reacts with water to afford the alkali-labile lesion. Thus, both products may be regarded as oxidation products of DNA. It is interesting that the same products can be formed from DNA

when BLM is activated with $Fe^{3+} + H_2O_2$, rather than $Fe^{2+} + O_2$ [16]. This has led to the suggestion that BLM activation for DNA cleavage involves the formation of a BLM-metal hydroperoxide intermediate that undergoes heterolytic O-O cleavage either prior to or concomitant with DNA oxidation.

Unlike the chemistry of small molecule oxidation/oxygenation, which seems likely to occur by bimolecular collision of activated BLM with its substrates, the oxidative transformation of DNA substrates by bleomycin seems to involve at least two steps, namely, substrate binding and H-atom abstraction. Bleomycin has been noted to affect DNA degradation in a sequence selective fashion at a subset of $^{5'}GC^{3'}$ and $^{5'}GT^{3'}$ sites. This selectivity may result from the binding of metallobleomycins with enhanced efficiency to certain sites in DNA, to variations in DNA microstructure which render certain C-4' H-atoms more readily amenable to abstraction, or both. The importance of the H abstraction step in contributing to the observed sequence selectivity of cleavage by BLM is underscored by the finding that H abstraction from different DNA sequences is associated with different isotope effects [17]. H-atom abstraction seems to be rate-limiting for DNA degradation; it appears likely that the facility of H-atom abstraction from a given site is an important determinant of the extent of cleavage at that site, especially under experimental conditions that involve cleavage at a limited number of DNA sites.

Clinical Use

Bleomycin is an important drug in the curative PVB and VAB combinations for testicular carcinomas and germ cell cancers of the ovary. It also is active in various curative protocols for Hodgkin's disease and non-Hodgkin's lymphomas. Bleomycin is active as a single agent and in combination regimens for squamous carcinomas from various sites, including the skin, head and neck, cervix, and genitalia.

Summary

BLMs are active anti-cancer drugs because their unique mechanism of action which is based on BLMs' structure and chemical characteristics. BLMs contain metal-binding domain, which is responsible for metal ion binding and oxygen activation, and DNA binding domain. BLM activation for DNA cleavage involves the formation of BLM-metal hydroperoxide intermediate and production of the superoxide radical and the very toxic hydroxyl free radical. BLMs break the C3' to C4' bond of deoxyribose in DNA strands and cause single- or double-strand breaks in DNA, with resulting chromosomal deletion and fragmentation.

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