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Bleomycin: an antitumor antibiotic

by

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Abbreviations:

BlMs, Bleomycins

dsB, double strand break

ESR, electron spin resonance

ssB, single strand break

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Abstract

Bleomycins (BLMs) comprise a family of glycopeptides that are antitumor agents. The major components of this family is bleomycin A_2 and B_2. The antitumor activity of bleomycin is due to its ability to cause cellular DNA breakage, a process that requires oxygen and a reduced transition metal, such as Fe(II). Bleomycin can cause pulmonary fibrosis, which is the major and most serious adverse side-effect in BLM uses. This paper will focus on the properties of BLM and the mechanism of BLM action. Finally, the pulmonary toxicity of BLMs is briefly introduced.

Introduction

Bleomycins (BLMs), a family of glycopeptide antitumor antibiotics, are widely used in the treatment of lymphomas, head and neck squamous cell carcinoma, and testicular carcinoma. They were first discovered by Umezawa and co-workers from cultures of *Streptomyces verticillus* in 1962 [1]. BLMs have been used as a chemotherapeutic agent in over 80 countries in the world [1]. Bleomycin A2 and B2 are the major components in this drug [1]. BLM exerts its antitumor function through its ability to induce DNA damage. BLM usually chelates an iron and further combines with an oxygen molecule to form its activated form. The activated Fe(II)BLM abstracts a hydrogen atom from C-4' position of deoxyribose on DNA and finally causes DNA single and double strand breaks [2]. Some single strand breaks may be repaired by the DNA repair system in cells before cells enter mitosis. However, part of the single strand breaks that are not repaired and DNA double strand breaks may cause cell chromosome aberrations and even cell death [2]. The most serious adverse side-effect of BLM in the clinic is its induction of lung injury [3]. This paper will focus on the properties of BLM and the mechanism of DNA damage by BLM. Also, the pulmonary toxicity of BLM will be briefly introduced.

Chemical properties of bleomycin

1.Structure of bleomycin

The structure of BLM was determined in 1978 [4]. BLMs have the same bleomycinic acid backbone with three major moieties. About 200 different BLMs have been known, most of them differ from one another at their terminal amine moiety. For

example, BLM A_2 contains a dimethylsulfonium propylamine as its terminal amine whereas BLM B_2 contains an agmatine [4]. BLMs A_2 and BLM B_2 are the two major forms in this drug. The structure of BLM comprises three key functional domains (Figure I) [5]. (1) The metal-binding domain, which consists of b-aminoalaninamide, pyrimidine, and b-hydroxyhistidine moieties, is responsible for metal-ion coordination and oxygen activation. Recent studies suggest that this domain also participates in DNA binding. (2) The DNA-binding domain is comprised of the bithiazole ring system and a positively charged carboxyl-teminal substituent. (3) The carbohydrate region is thought to aid in membrane permeability and selective tumor-cell recognition. The structure of the amino acid that acts as a "linker" between the metal-binding and DNA-binding domains affects the efficiency of DNA degradation and the degree of the antitumor activity [5].

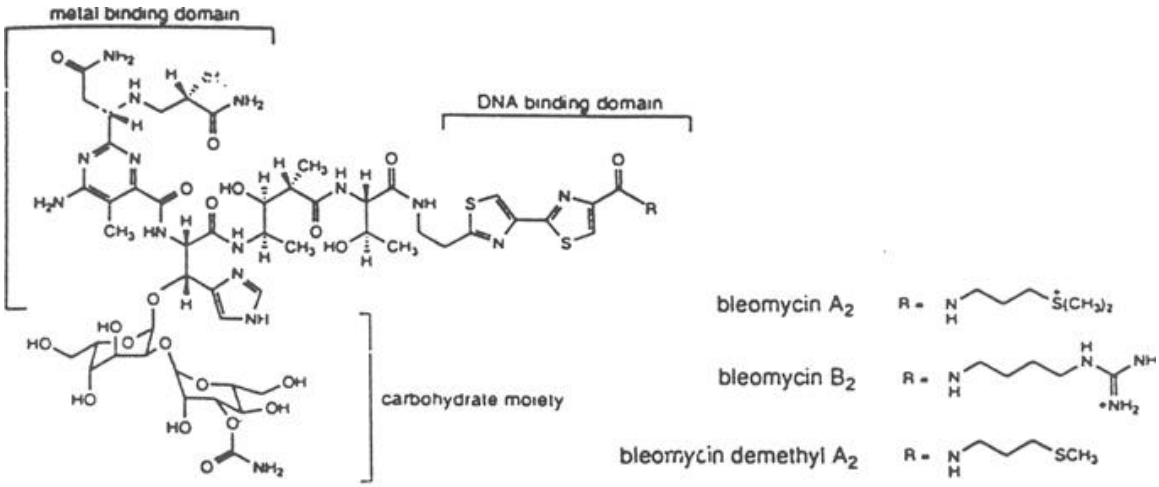

Figure I. Structure of representative bleomycin group antibiotics [5].

2. Metal-complex of BLM

The BLMs can chelate a wide variety of metals including Fe, Cu, Co, Zn and Mn [6]. The iron complexes of BLMs are probably the most important for the effects of

BLMs *in vivo.* BLM is a bifunctional compound: one function is to bind DNA and another one is to react with DNA [6]. Figure II shows that Fe(II)BLM is a square pyramidal structure with a basal plane that contains a secondary amine nitrogen, a N-4 pyrimidine ring nitrogen, which is the deprotonated peptide bond nitrogen of the histidine region and a histidine imidazole nitrogen. The fifth axial donor has been assigned to the a-amino nitrogen of the β-aminoalanine moiety of BLM and the sixth axial coordination site is occupied by exogenous oxygen [6]. If oxygen reduces, it will form both hydroxyl and superoxide radicals based on spin-trapping data [7]. The positively charged terminal amino group of bleomycin is electrostatically attracted to the negatively charged phosphate group of DNA, and the planar bithiazole group is intercalated into the double-stranded DNA [6].

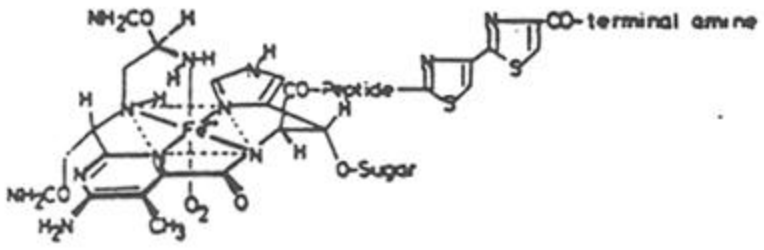

Figure II. Postulated structure of Fe(II)-bleomycin complex [6].

Mechanisms of DNA degradation with BLM

1. Formation of activated BLM

It has been demonstrated that Fe(II) and oxygen are essential cofactors for BLM mediated DNA degradation. The activated BLM is believed to be the DNA attacking species. The activated BLM is formed in several distinct steps. As shown in the

following reactions, Fe(II)BLM is unstable in the presence of dioxygen and reacts rapidly with it to form a ternary complex, HO_2-Fe(III)BLM (activated FeBLM)[7].

$$Fe(II) + BLM \rightleftharpoons Fe(II)BLM \quad (1)$$

$$2Fe(II)BLM + O_2 + H^+ \rightleftharpoons HO_2\text{-}Fe(III)BLM + Fe(III)BLM \quad (2)$$

$$Fe(III)BLM + H_2O_2 \rightleftharpoons HO_2\text{-}Fe(III)BLM + H^+ \quad (3)$$

The activated FeBLM then reacts with the DNA backbone and generates a C4' carbon radical on deoxyribose that undergoes further reactions to produce strand cleavage [7].

2. Binding of activated BLM to DNA

Experimental evidence suggests that the minor groove of the DNA helix is the binding site for BLM [8]. The moiety on BLM that is responsible for binding to DNA is the bithiazole moiety and its associated amine and/or sulfonium residue. BLM-mediated DNA damage is sequence-specific involving mainly pyrimidine residues in a 3'-position to G such as G-C and G-T [9]. There is evidence that also shows that the metal binding domain contributes to both DNA binding and sequence specificity [9]. It is now believed that the bithiazole is primarily responsible for sequence-independent binding activity, while the metal-binding domain determines the sequence specificity.

3. BLM mediated DNA damage and its mechanism

BLM-mediated DNA damage includes single strand breaks (ssB), double strand breaks (dsB) and release of free bases without scission. The ssB and dsB occur on limited sites in DNA. Some single strand breaks may be repaired by the DNA repair system in cells before cells enter mitosis. However, dsB, the release of free bases and

part of ssB that are not repaired may cause cell chromosome aberrations and even cell death [2].

Once activated Fe(II)BLM is formed, BLM begins to initiate DNA damage by abstracting the hydrogen atom at the C4' position of deoxyribose [10]. DNA degradation splits into oxygen-dependent and oxygen-independent pathways, (A) and (B) respectively, see Figure III [10].

Under anaerobic conditions, degradation results in the liberation of free bases and the formation of an oxidatively damaged sugar that cleaves alkali-labile compound [10]. In the presence of oxygen, degradation results in the cleavage of the deoxyribose C3'-C4' bond with the release of a base propenal, the DNA chain is broken with oxidation of the C3' and release of a 5'-phosphate ester, and a 3'-phosphoglycolate group (Figure IV) [11].

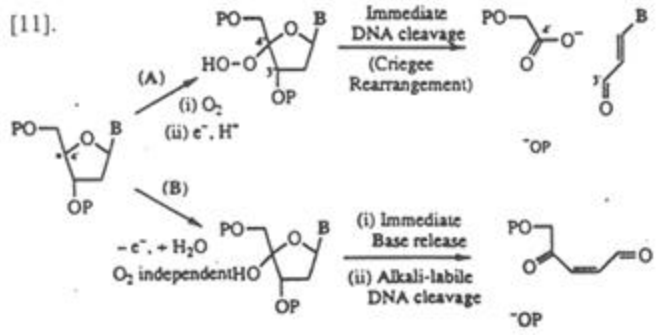

Figure III. Two pathways to DNA cleavage are induced by Fe(II)BLM [10]

The mechanism by which BLM is able to abstract C4'-hydrogen from deoxyribose on DNA is debatable. Studies using ESR confirmed that the oxidation of Fe(II)BLM produced a small amount of hydroxyl radical under the condition of excess of BLM. One model [11], which involves the formation of hydroxyl radicals, proposes that the O_2•Fe(II)•BLM complex undergoes hemolytic O-O bond cleavage by a reducing

agent to produce the equivalent of a hydroxyl radical and (˙OH)Fe(III)•BLM. Alternately, (˙OH)Fe(III)•BLM is directly formed from Fe(III)•BLM by hydrogen peroxide. The (˙OH)Fe(III)•BLM is thought to be able to abstract the C4' hydrogen from DNA. However, this model has been criticized. Evidence has shown either that BLM can induce extensive unscheduled DNA synthesis in the absence of formation of oxygen radicals [12]. Or that the 10-hydroperoxy-8, 12-octadecadienoic acid-activated-BLM does not result in the degree of DNA damage that is correlated with the consumption of the peroxide [12]. Instead, a perferryl species, which is produced by heterolytic O-O cleavage, perhaps is more likely to be responsible for C4'-hydrogen abstraction [12].

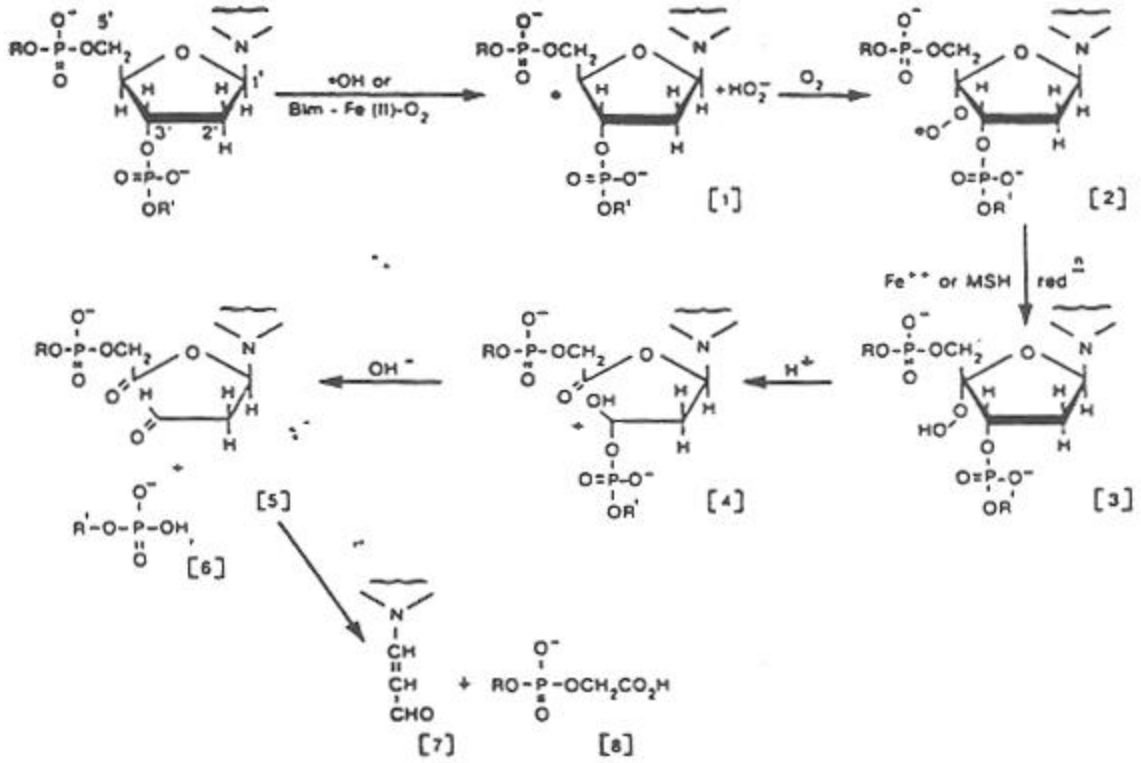

Figure IV [11]. Mechanism of DNA degradation induced by the activated BLM-Fe(II)-O_2 complex. The activate complex, initially abstracts a hydrogen atom from the 4' position to produce the unstable intermediate [1] that decomposes in the presence of oxygen to produce the free base propenal [7], leaving a 3'-phosphoglycolate ester [8] and a 5'-phosphate [6] at the free ends of the broken DNA strand [11].

Pulmonary toxicity of BLM

Minor adverse reactions to BLM include nausea, vomiting, fever, and occasional allergic reactions. However, the major clinical obstacle for the expanded use of BLM is its propensity to cause pulmonary fibrosis. Mortality from pulmonary toxicity occurs in 1-2% of patients treated [3] and about 2%-3% of patients suffer nonlethal pulmonary fibrosis [3].

The reason for pulmonary toxicity of BLM is still unclear. It seems that alveolar macrophages play a central role in the pulmonary cytokine network and they mediate the initiation of BLM induced fibrosis. Treating macrophages with BLM can increase the secretion of interleukin β_1, tumor necrosis factor α, and prostaglandin E_2 [13]. BLM also stimulates the production of other lymphokines, such as macrophage fibroblast growth factor and transforming growth factor β, both of which can cause the development of an inflammatory response that may lead to fibrosis [13]. In addition to macrophage involvement, endothelial cells are an important *in vivo* source of transforming factor β and can also release a greater amount of this factor when the cells are coincubated with BLM.

Summary

Bleomycins (BLMs) are a family of glycopeptide antitumor antibiotics. Bleomycin-induced cell killing is due to its ability to cause cellular DNA breakage. This process involves an iron- and oxygen-dependent redox reaction. In the clinic, the major side-effect for using BLM is its propensity to cause pulmonary fibrosis.

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