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A Wider Scope for the Antibiotic and Anticancer Drug Bleomycin

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ABSTRACT

Bleomycin is classified as a glycopeptide antibiotic initially discovered in the late 1950s, and used for treating a limited set of cancers. The structure and modes of action of bleomycin have been studied and established. It is believed that the primary antitumor effect of bleomycin is due to its ability to damage DNA, however the drug has the potential to destroy other targets. While we have focused previously on factors such as uptake transporters that could limit the genotoxicity of bleomycin, this mini-review will provide an overview of the drug actions on various macromolecules. There is a possibility that bleomycin may have broader clinical applications by exploring its effects on other cellular targets such as RNA.

KEYWORDS

Cell wall, Plasma membrane; RNA; DNA damage; Transporters

1. INTRODUCTION

Bleomycin is a hydrophilic antibiotic isolated from the culture medium of *Streptomyces verticillis* [1,2]. It comprises many species differing at the terminal amine, with bleomycin-A2 representing the most abundant form [2-5]. By early sixties, it had been shown that bleomycin can suppress the growth of tumors in animal models, and likewise substantially decrease the size of human tumors [6-10]. Bleomycin was proposed to mediate cell killing by damaging the DNA [11,12]. Further independent studies demonstrated that bleomycin triggers the induction of lysogenic phage in bacteria, a consequence of DNA damage [13-18]. In addition, it induces mitotic recombination and alters the genome in many organisms including the budding yeast *Saccharomyces cerevisiae*, *Aspergillus*, and *Drosophila* [13-18]. In human lymphocytes, bleomycin was shown to induce micronuclei formation and chromosome aberrations [19]. From the above findings, it is clear that bleomycin has the potential to act as a chemotherapeutic agent by damaging the DNA [20-23]. Importantly, bleomycin can also cause severe damage to RNA, and in light of the COVID-19 pandemic that began in December of 2019, there is renewed interest in this drug as an antiviral agent [24].

Bleomycin is routinely used in almost all clinics in the world as blenoxane, which consists of several isomers that include bleomycin-A2 and bleomycin-B2, and many additional minor species such as bleomycin-A5 [4]. Blenoxane is used only in combination therapy with other antineoplastic agents such as etoposide [4,25,26]. It is most effective against lymphomas, testicular carcinomas, and squamous cell carcinomas [27,28]. In comparison to other antineoplastic drugs, bleomycin does not appear to cause myelosuppression by decreasing the cells

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responsible for immunity [28,29]. At least half of the drug is cleared from the blood by the renal system within 2 hours to 4 hours, except for patients with impaired kidney function [29]. One key limitation of bleomycin is that at high doses (i.e., >400 units or ~235 mg), it can induce pulmonary fibrosis, a condition characterized as a diffuse disease of the lung parenchyma that can cause pulmonary insufficiency leading to fatal hypoxemia [30,31]. It is believed that the bleomycin-induced pulmonary fibrosis is triggered by lipid peroxidation [32,33]. Another common factor that limits the clinical application of bleomycin is tumor resistance [28]. Recent studies provide strong evidence that this resistance can be accounted for by a decrease in drug uptake [34-38]. We have previously documented the discovery of an uptake transporter, Agp2, which is responsible for allowing the entry of the drug into yeast cells. Cells lacking Agp2 were extremely resistant to the toxic effects of bleomycin [39,40]. It was this seminal study that refutes the notion that bleomycin gains entry into cells by diffusion and leading to the discovery of additional transporters from other organisms [41]. In this review, our focus is to the highlight the targets of bleomycin with the aim that there will be renewed interest to investigate this drug for its potential antiviral properties.

2. THE FUNCTIONAL DOMAINS OF BLEOMYCIN

The structure of bleomycin consists of four functional domains, including a metal binding domain, a DNA binding domain, a linker region that connects both domains, and the carbohydrate moiety (Figure 1) [42,43]. The metal domain, which also binds to molecular oxygen, is responsible for the anti-cancer properties of bleomycin. This domain has a relax specificity for the types of metal ions it can accommodate, as it can bind to both redox-active metal ions such as iron and copper and the non-redox ones such as zinc, cadmium and cobalt, and the latter forms a stable complex with bleomycin [44-49]. The redox-active metal ion serves two roles in bleomycin-induced genotoxicity, i.e., facilitating contact between bleomycin and the DNA and activating oxygen to produce a reactive radical species [20,45,46,50-52]. Thus, to enhance the production of DNA lesions reduced iron is used in clinical preparations of active bleomycin [52,53].

The DNA binding domain of bleomycin carries a bithiazole group that intercalates with the DNA and performs sequence-selective DNA cleavage (Figure 1) [22,54,55]. Some species of bleomycin, such as bleomycin-A5, carry a polyamine moiety in the DNA binding domain resulting in a new class of anti-cancer agent referred to as polyamine analogue (Figure 1) [56]. The role of the other regions of bleomycin remains unclear, although loss of the carbohydrate moiety from bleomycin does not affect its ability to cleave DNA, but may interfere with its entry into cells [57].

3. TYPES OF DNA LESIONS INDUCED BY BLEOMYCIN

We have documented that bleomycin can enter mammalian cells through an active transport pathway, and reaches the nucleus to produce specific types of DNA lesions [39,40,58,59]. Bleomycin can bind to reduce iron (Fe II) and in the presence of molecular oxygen it becomes activated [60]. The activated bleomycin (Blm-Fe(II)-O2) complex is a powerful oxidant, abstracting a hydrogen atom from the 4'-carbon of deoxyribose producing an unstable sugar carbon-radical and a single electron reduced form of activated bleomycin (Blm-Fe(III)-OH•), which can propagate its attack on DNA (Figure 2) [61-64]. The resulting unstable sugar can be rearranged to generate at least four types of oxidative DNA lesions (Figure 2). These lesions are very similar to those generated by ionizing radiation, and include: (i) Oxidized apurinic/apyrimidinic (AP) sites, lacking template information for DNA polymerase [58,65], (ii)

DNA single strand breaks where the 3'-ends are terminated with a portion of the deoxyribose ring to form 3'-phosphoglycolate (3'-PG) which cannot support DNA synthesis (Figure 2) [65,66]. The remaining portion of the fragmented sugar can undergo secondary reactions to form additional base adducts [65-70], and (iii) Bi-stranded DNA lesions that are generated at certain sequences, such as CGCC, when the Fe.bleomycin complex induces an AP site on one strand, while creating a directly opposing strand break on the complementary strand [49,71-73]. The bi-stranded lesions can be converted to double strand breaks following spontaneous cleavage of the AP site by primary amines (e.g., histone amine) in vivo [71-73].

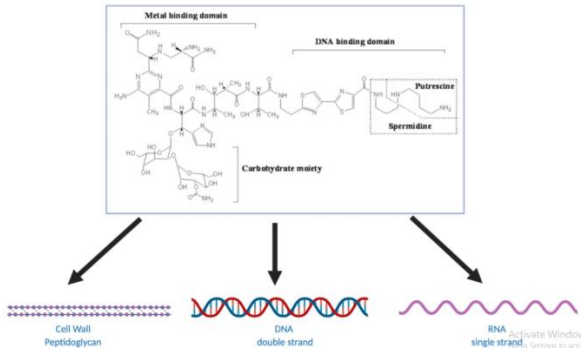

Figure 1: Structure of bleomycin, depicting the three active domains, and its cellular targets.

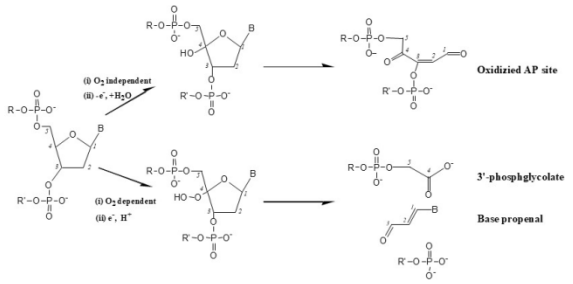

Figure 2: Structure of bleomycin-induced DNA lesions. Production of the various types of bleomycin-induced lesions is dependent on oxygenation conditions. In the absence of oxygen, bleomycin produces primarily oxidized apurinic/apyrimidinic (AP) site, while in the presence of oxygen it generates mostly DNA strand breaks, such as 3'-phosphoglycolate.

The redox status of the cells plays an important role in the types of lesions that are generated by bleomycin [74-77]. In the presence of oxygen, bleomycin produces primarily DNA strand breaks, but under low oxygen tension it forms mainly AP sites (Figure 2) [58,65,71,78]. In addition, at high concentrations, bleomycin releases all four bases from DNA in the order of preference thymine > cytosine > adenosine > guanine [58,62,79]. At lower concentrations, bleomycin exhibits significant base sequence specificity. Although bleomycin cuts mixed sequence DNAs with a disposition for GC=GT>GA>>GG, it efficiently cleaves regions of (AT)n•(TA)n and hardly at (ATT)n•(TTA)n, (ATT)n•(AAT)n, (AC)n•(GT)n and (A)n•(T)n raising the possibility that AT rich regions of the genome are more susceptible to lesions formed by bleomycin [80-82]. The structure of DNA also plays a role in the outcome of bleomycin-induced DNA lesions and a recent study confirmed that 5'-GT, 5'-GT*A, and 5'-T/CGT*A were predominant sequences cleaved by the drug [43,83]. DNA that is pre-exposed to other DNA damaging agents, such as cisplatin, alters the pattern of lesions produce by bleomycin [84-86]. Thus, the clinical application of bleomycin in combination with other DNA damaging agents is likely to produce irreparable DNA lesions.

The DNA lesions induced by bleomycin are mutagenic [87-91]. Introduction of bleomycin-treated vectors into mammalian cells, followed by recovery, revealed that the vectors contain high levels of base substitutions and single-base deletions [87,88]. These modifications are the results of misincorporation of nucleotides by DNA polymerase at unrepaired oxidized AP sites, and incorrect repair of bi-stranded DNA lesions [87,88]. Therefore, normal cells of a cancer patient exposed to bleomycin must rely on enzymes to efficiently repair bleomycin-induced DNA lesions to prevent the production of lethal mutations that can lead to toxic side effects and secondary tumors. We have previously discussed the enzymes that are involved in processing bleomycin-induced DNA lesions and the consequences of cells lacking these enzymatic functions [92,93].

4. BLEOMYCIN ACTIONS ON RNA

Bleomycin can also attack different species of RNAs including transfer RNAs, ribosomal RNA, RNA present in RNA•DNA heteroduplex, as well as viral RNA such as the HIV-1 reverse transcriptase mRNA [94-99]. The drug exploits the same oxidative mechanism as that used for cleaving DNA to incise RNA [100, 101]. Bleomycin-induced cleavage of RNA occurs preferentially at 5'-GU-3' sequences in a manner analogous to the site-specific cleavage that occurs in DNA at 5'-GT-3' [94,100]. It is noteworthy that not all RNA molecules such as E. coli tRNA-Tyr and yeast mitochondrial tRNA-Asp, can be cleaved by bleomycin, suggesting that RNA structure plays an important role in the recognition and cleavage processes [94,100]. Another key difference between the cleavage of RNA and DNA is that double stranded RNA is not incised by bleomycin [101]. Moreover, significantly less RNA is cleaved by bleomycin as compared to DNA [94]. In addition, the cleavage of RNA, and not DNA, is inhibited with 0.5 mM Mg^{2+} ions, which is required to maintain RNA structure and function [95]. It is suggested that the Mg^{2+} ions prevent bleomycin from accessing the cleavage site [95]. The selectivity of bleomycin to destroy certain RNAs, even in the excess of non-substrate RNAs, suggests that unique RNA species could be targeted for destruction by bleomycin. It is believed that the specificity of RNA cleavage by bleomycin can be harnessed to eliminate virulent RNA viruses despite the evidence that DNA might be the most suitable target [94,95,101].

Since many viruses such as the hepatitis C virus upon infection of cells trigger the production of reactive oxygen species that can damaged the DNA and inhibit the DNA damage response pathway, it seems likely that bleomycin could perform multiple roles by destroying the viral RNA and hypersensitized the infected cells by further damaging the DNA (Figure 1). It seems plausible that virus-infected cells treated with bleomycin are at a disadvantage and can be easily eliminated by DNA damage-induced apoptosis [102,103]. Moreover, bleomycin has been shown to inhibit the replication of hepatitis C virus and sensitizes other viruses such as HIV to antiviral agents, which raise the possibility that this drug may have significant antiviral properties [104,105].

5. CELL SURFACE TARGETS

Bleomycin can also attack the integrity of the cell wall of microbes [106,107]. It destroys the cell wall via oxidative damage to the sugar, as the sugar constituents (glucans, mannoproteins and chitin) of the cell wall have a stereochemistry at the C-5 position that is similar to the C-4 position of the deoxyribose moiety of DNA. Damage of the cell wall by bleomycin can expose the protoplast, which is osmotically fragile leading to the disruption of plasma membrane and subsequent cell death [106-108]. Bleomycin causes damage to the plasma membrane by triggering lipid peroxidation, and this may constitute the initiation process of bleomycin-induce pulmonary fibrosis [32,109,110].

6. PERSPECTIVES

During the last two decades, we have extensively exploited the budding yeast Saccharomyces cerevisiae to identify how this organism mount a response to bleomycin. We have shown in several studies that bleomycin can damage the DNA and required at least two major DNA repair pathways, the base-excision and the recombinational DNA repair pathways, to process the damaged DNA. While we had envision in the late 1990s that upregulation of DNA repair mechanisms would predominantly account for tumor resistance to bleomycin, it turns out that the uptake of the drug into bacteria, yeast and human cells is a key factor that determines resistance (Figure 3) [39,40]. We have first reported the identification of an uptake transporter called Agp2 in yeast, which modulates the sensitivity of cells to bleomycin (Figure 3). Cells lacking Agp2 or

overexpressed the transporter were either extremely resistant or hypersensitive to bleomycin, respectively [39,40]. We subsequently reported a similar observation by the hCT2 transporter in human cells (Figure 3) [59]. However, it remains to be defined whether tumors such as testicular and ovarian cancers that are resistant to bleomycin are due to defects in hCT2 function(s).

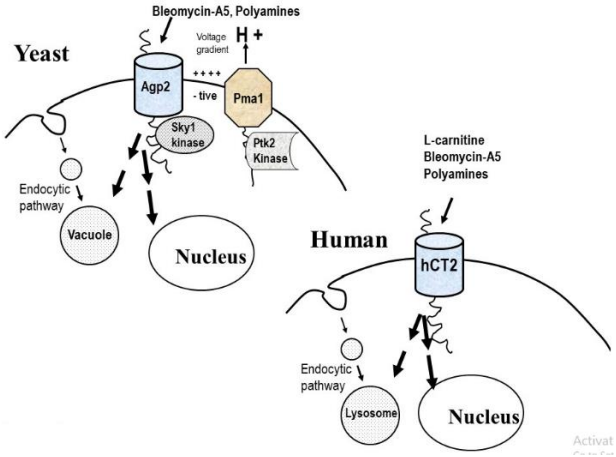

Figure 3: A model illustrating the transport and detoxification pathway of bleomycin in yeast and human cells. In yeast, the drug enters the cell via the transporter Agp2, and its activity is regulated by the kinases Ptk2 and Sky1. Following uptake, bleomycin is channeled to the nucleus to destroy the DNA, and to the vacuole for detoxification. In humans, the hCT2 transporter can mediate uptake of bleomycin into cells. Defects in these transporters confer resistance to bleomycin.

Besides the above notable discoveries, what remains uncertain is whether other properties of bleomycin namely the ability to oxidize the plasma membrane to form lipid peroxidation products or its ability to cleave RNA would have roles in clinical applications (Figure 1). The observations that bleomycin can inhibit replication of hepatitis C virus and sensitize HIV to antiviral agents strongly suggest that the next plausible future direction is to investigate bleomycin as an antiviral agent. In light of the latest world pandemic, and the hunt for new drugs as antiviral agents, it seems that bleomycin offers some properties that might satisfy this requirement. It would be worthwhile to test bleomycin in combination with other antiviral drugs such as Remdesivir in an attempt to curtail the virulence of COVID-19.

7. ACKNOWLEDGEMENT

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8. CONFLICT OF INTEREST STATEMENT

The authors declare that they have no conflicts of interest with the contents of this article.

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