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Review

DNA and chromosome damage induced by bleomycin in mammalian cells: An update

Alejandro D. Bolzán[a,b,*], Martha S. Bianchi[a]

[a] Laboratorio de Citogenética y Mutagénesis, Instituto Multidisciplinario de Biología Celular (IMBICE, CICPBA-UNLP-CONICET La Plata), calle 526 y Camino General Belgrano, B1906APO La Plata, Buenos Aires, Argentina

[b] Universidad Nacional de La Plata, Facultad de Ciencias Naturales y Museo, calle 60 y 122, La Plata, Buenos Aires, Argentina

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ABSTRACT

Bleomycin (BLM) is an antibiotic isolated from *Streptomyces verticillus*. It has radiomimetic actions on DNA thus it has been widely used in clinical chemotherapy for the treatment of different types of cancer, including head and neck tumors, lymphomas, squamous-cell carcinomas and germ-cell tumors. Because of this, the study of BLM genotoxicity is of practical interest. This antibiotic is an S-independent clastogen and an agent that generates free radicals and induces single- and double-strand breaks in DNA. In the present review, we will summarize our current knowledge concerning the DNA and chromosome damage induced by BLM in mammalian cells, with emphasis on new developments published since 1991.

1. Introduction

Bleomycin (BLM, CAS No. 9041-93-4) is a radiomimetic antitumor antibiotic first isolated from *Streptomyces verticillus* [1–3]. Actually, this drug belongs to bleomycins (BLMs), a family of glycopeptide-derived antibiotics, which includes bleomycinic acid, BLM A2 and BLM B2, among others [4,5]. BLMs are structurally and biosynthetically related to phleomycins and tallysomycins [5].

BLM is widely used in clinical chemotherapy for the treatment of different types of cancer, namely testicular cancer, lymphoma, lung cancer, cervical cancer and cancers of the head and neck [4,5]. Its clinical formulation Blenoxane is a mixture of components, primarily bleomycin A2 and B2 [2,4,5]. Unlike most anticancer drugs, BLM does not cause myelosuppression, but early development of drug resistance and cumulative lung fibrosis are the major limitations of its use in chemotherapy [6,7]. BLM resistance seems to be associated with reduced DNA damage after BLM exposure, resulting in reduced G2/M arrest and reduced apoptosis [7].

The toxic effects of BLM are thought to be related to its ability to mediate both single-stranded and double-stranded DNA damage, which requires the presence of specific cofactors (a reduced transition metal (Fe(II) or Cu(I)), oxygen and a one-electron reductant) to generate what is called “activated” BLM [4,5]. This chemical species can destroy itself, oxidize lipids, hydrolyze amide bonds of proteins or initiate cleavage events on RNA and DNA molecules – in the latter case through the production of free radicals by activated BLM bound to DNA – that react rapidly and non-specifically with any molecule they encounter [4]. Moreover, BLM can be metabolically inactivated in normal and tumor tissues by an enzyme called BLM hydrolase [5].

Because of the wide use of BLM for the treatment of cancers, the study of its genotoxicity in mammalian cells is of practical interest. As we will see in the next sections, this antibiotic tests positive in the great majority of genotoxicity assays in mammalian cells, including chromosomal aberrations (CAs), micronucleus (MN) and comet assay. In the present review, we will summarize our current knowledge concerning the DNA damaging and clastogenic effects of BLM on mammalian cells, with emphasis on new developments reported in the last 26 years, since the last general review on the genotoxicity of BLM was published by Povirk and Finley Austin in 1991 [2].

Abbreviations: BLM, bleomycin; CAs, chromosomal aberrations; MN, micronucleus; FISH, fluorescent *in situ* hybridization; mtDNA, mitochondrial DNA; SCEs, sister-chromatid exchanges; ITSs, interstitial telomeric sequences; γ-H2AX, phosphorylated histone H2AX; PNA, peptide nucleic acid; CHO, Chinese hamster ovary cells; CHE, Chinese hamster embryo cells; ICE, incomplete chromosome elements; TSSs, DNA cleavage transcription start sites; MMR, mismatch repair; GSH, glutathione; BME, β-Mercaptoethanol; CYST, cysteine; CSM, cysteamine; DTT, ditiothreitol

* Corresponding author at: Laboratorio de Citogenética y Mutagénesis, Instituto Multidisciplinario de Biología Celular (IMBICE, CICPBA-UNLP-CONICET La Plata), calle 526 y Camino General Belgrano, B1906APO La Plata, Buenos Aires, Argentina.
E-mail address: abolzan@imbice.gov.ar (A.D. Bolzán).
URL: http://mailto:adbolzan64@gmail.com (A.D. Bolzán).

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2. DNA damage induced by BLM in mammalian cells

2.1. General remarks

For a review of the chemistry of DNA damage induced by BLM we refer the reader to the excellent articles by Chen and Stubbe [4] and Galim et al. [5], published about a decade ago. In this section of the present review, we will focus on the types of DNA damage induced by BLM on mammalian cells, putting emphasis on the latest developments on this subject.

Several studies have shown that BLM induces base damage, single and double-strand breaks and apurinic/apyrimidinic sites in the DNA molecule (see [2–4] for review). The critical lesions in the cytotoxic effect of BLM are DNA double-strand breaks [2–4], and recent evidence by Chen et al. [8] shows that multiple binding modes of a single BLM molecule can lead to DNA double-strand breaks, that this damage can occur using one or two BLM molecules, and that the ratio single-strand:double-strand breaks varies between the different BLMs. Moreover, Liu et al. [9] using mouse embryo fibroblasts with distinct polymerase β expression levels, showed that BLM-induced DNA damage can be repaired through the base excision repair pathway, and that the absence of this enzyme (in polymerase β deficient cells) promotes oxidative DNA/chromosome damage and gene mutation, which contributes to BLM hypersensitivity. Furthermore, Liddle et al. [10] using Chinese hamster ovary cells (i.e., CHO cell line), showed that BLM-induced γ-H2AX foci (the phosphorylated form of the histone H2AX, which occurs in response to DNA double-strand breaks formation) map preferentially to replicating domains in interphase nuclei.

It is well-known that BLM intercalates G-rich tracts of DNA and induces strand breakage by preferential attacking of pyrimidine nucleotides that adjoin the guanosyl-3-phosphate at the site of BLM-DNA binding [4]. Several lines of evidence suggest that DNA damage induced by BLM in living cells is modulated by different factors, including chromatin structure [11–13], DNA repair [12–14], BLM hydrolase [15], antioxidant enzymes [16,17] and thiol-containing compounds [18–23]. Moreover, actively transcribed genes are more susceptible to BLM than silenced genes [11] and BLM-induced DNA damage is cell-cycle dependent [24]. In effect, in synchronized Chinese hamster cells, BLM caused 2–3 times fewer DNA double-strand breaks in S-phase cells than in G1 or G2/M phase cells, i.e., during S-phase BLM produces DNA damage, but in a lesser extent than in G1 or G2 phases [24]. This observation is in good agreement with early studies in mammalian cells (HeLa and CHO) showing that BLM does not directly interfere with DNA replication (i.e., it does not inhibit the initiation and completion of DNA synthesis) [25,26].

The more recent findings on the DNA damaging effects of BLM on mammalian cells refer mainly to those ones made using the comet assay to detect DNA strand breaks, and molecular biology techniques to analyze the effect of this compound on telomeric and mitochondrial DNA (mtDNA) and to determine the genome-wide pattern of DNA cleavage by BLM. Next, we will briefly discuss these findings.

2.2. BLM-induced damage on telomeric and mitochondrial mtDNA. Other studies on BLM-induced DNA damage in mammalian cells

More than a decade ago, Arutyunyan et al. [27] applied for the first time the Comet-FISH technique (i.e., single cell gel electrophoresis or comet assay in combination with fluorescent *in situ* hybridization or FISH) with a telomere-specific peptide nucleic acid (PNA) probe, to analyze the damage induced by BLM (and also mytomicin C) on telomeric DNA. These authors found that both anticancer drugs induce fragmentation (breaks) in telomere-associated DNA in human lymphocytes. However, BLM and mytomicin C induced DNA breaks in the DNA of or adjacent to telomeric repeats were found to be proportional to that of total DNA, which suggests random induction of DNA breaks by these two antibiotic compounds. The same year, Milić and Kopjar [28] performed a similar study but using the alkaline comet assay alone and found that both drugs and their combination induce a significant DNA damage, showing a synergetic effect in these cells, although BLM alone induced the highest level of damage. A year later, Arutyunyan et al. [29], using Comet-FISH, also showed that, in human lymphocytes, BLM induces telomere DNA damage. The induction of telomere DNA damage by BLM in mammalian cells was confirmed by Hovhannisyan et al. [30], who analyzed the effect of these drugs in normal human leukocytes and three transformed cell lines (HT1080, CCRF-CEM and CHO) using Comet-FISH. It was shown that telomeres in CHO and CCRF-CEM cells were about 2–3 times more sensitive towards BLM than global DNA, while in HT1080 telomeres were less fragile than total DNA. Moreover, these authors found significant differences between the above cell lines with respect to quantitative head/tail distribution of telomeric signals after BLM exposure: while a large number of telomeric signals of various sizes were found in CHO cells, very small signals were detected in the comets of HT1080 and CCRF-CEM lines. This is probably due to the fact that CHO cells contain large blocks of interstitial telomeric repeats [31], while human tumor cell lines have short telomeres [32]. A further analysis of the studies of DNA damage by BLM and other anticancer drugs using Comet-FISH can be found in the review article of Glei et al. [33]. Recently, Liu et al. [34,35], studied the effect of BLM and other anticancer drugs on telomeres of a mouse spermatogonial cell line and rat male germ cells (from Brown Norway rats). The co-localization of telomere and γ-H2AX signals after FISH and immunofluorescence, respectively, observed in these cells, indicated that BLM damages telomeric DNA of germ cells.

A few years ago, Nguyen and Murray performed a pair of studies to determine the effect of BLM on human telomeric DNA using DNA sequencing [36,37]. These studies showed that human telomeric DNA sequences are a major target for this anticancer drug [36,37]. They examined the DNA sequence specificity of BLM in a target DNA sequence containing 17 repeats of the human telomeric sequence and other primary sites of BLM cleavage and found that BLM cleaved primarily at 5′-GT in the telomeric sequence 5′-GGGTTA [36,37]. The telomeric region constituted 57% of the 30 most intense BLM damage sites in the DNA sequence examined, which indicates that telomeric DNA sequences are a major target for BLM damage. More recently, Chung and Murray [38], using end-labeled DNA and capillary electrophoresis, analyzed the DNA sequence specificity of BLM in two human mtDNA sequences. This compound was found to cleave preferentially at 5′-TGT*A-3 DNA sequences (where * is the cleavage site). Previously, Yeung et al. [39] analyzed the mtDNA damage induced by BLM in acuted myeloid leukemia cells and reported that this compound damaged mtDNA at concentrations that induced cell death.

On the other hand, the alkaline comet assay was also employed to analyze the rejoining kinetics of BLM-induced DNA damage in human lymphocytes [40]. These authors detected early (0–30 min) events in the induction of single-strand breaks in the lymphocytes of 45 individuals, and showed that, after DNA damage induction, the fastest return to the background level occurred in 5 min, whereas the lowest return took approximately 30 min [40]. Besides, the early rejoining kinetics of single-strand breaks showed multiple patterns, depending on the individual analyzed. Moreover, Weng et al. [41], analyzed the DNA damage induced by BLM and H_2O_2 in different subpopulations of human white blood cells using the comet assay and found that they differ in their sensitivity to these compounds, B-cells showing the highest sensitivity to BLM.

2.3. Studies on the genome-wide pattern of DNA cleavage by BLM in human cells

In 2014, Murray and coworkers [42] investigated the genome-wide pattern of DNA cleavage by BLM at transcription start sites (TSSs) of actively transcribed and non-transcribed genes in human HeLa cells, using next-generation DNA sequencing. They found that actively