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Bleomycin induces short-term telomere fragility in Epstein-Barr virus-transformed human lymphoblastoid cells

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ABSTRACT

The induction of telomere dysfunction-related chromosomal aberrations by the radiomimetic antibiotic bleomycin (BLM) was studied in human lymphoblastoid cells immortalized with the Epstein-Barr virus (EBV). To this end, an EBV-induced lymphoblastoid cell line (T-37) was exposed to increased concentrations of BLM (10–100 μg/mL) for 2 h at 37ºC, and telomere aberrations were analyzed 24 h (first mitosis) after treatment using PNA-FISH with pan-telomeric plus pan-centromeric probes. Telomere signal duplications (TSD) increased significantly in BLM-exposed cells ($p < 0.01$), although the concentration-response relationship was non-linear. Most of the induced TSD (95–99 %) were of chromatid-type. No induction of telomere signal loss, telomere fusions or telomere associations by BLM was observed in T-37 cells. These findings show that BLM induces short-term telomere dysfunction in EBV-transformed human lymphoblastoid cells in the form of TSD (which implies telomere fragility) and suggest that these effects mainly occur during the G2 stage of the cell cycle. The persistence of this type of aberrations in the long-term in EBV-induced lymphoblastoid cells and other human cells exposed to BLM may be of medical relevance. Telomere fragility induced by BLM could promote genomic instability, which might contribute to the development of secondary tumors in patients undergoing chemotherapy based on this compound. Consequently, our study raises concerns about the potential long-term genomic effects of BLM in treated patients and suggests that the analysis of TSD could be a useful biomarker for detecting BLM-induced telomere dysfunction in human cells.

1. Introduction

Bleomycin (BLM, CAS No. 9041–93–4) is a radiomimetic compound with antibiotic, antitumor, and clastogenic properties, first isolated from *Streptomyces verticillus* [1]. 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 [2,3]. Despite of its antineoplastic properties, BLM is also genotoxic, producing both DNA and chromosome damage [1,3]. For the above reasons, the study of the genotoxic effects of BLM in human cells is of clinical interest. Although the chromosome damage induced by BLM has been extensively investigated in human and non-human cells (see [3] for reiew), data on the clastogenic effects of this compound on human telomeres are scarce [4,5].

Telomeres protect chromosomes from degradation, recombination, or fusion [6]. Therefore, to preserve telomere homeostasis is vital to maintain genome stability. Dysfunctional or critically short telomeres trigger the DNA damage response, potentially leading to genomic instability, a common event in cancer cells [6–9]. In fact, unstable telomeres lead to chromosome instability, further promoting genomic instability [6,10,11]. Therefore, analysis of BLM-induced telomere instability is relevant to understand genomic instability induced in patients undergoing chemotherapy based on this compound.

Telomere instability may appear after one or more chromosomes lose

Abbreviations: BLM, bleomycin; EBV, Epstein-Barr virus; FISH, fluorescence in situ hybridization; PNA, peptide nucleic acid; TSL, telomere signal loss; TSD, telomere signal duplication; TF, telomere fusions; TA, telomere associations.

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one or both ends (giving rise to a true telomere loss) or due to telomere dysfunction (when the telomere loses its end-capping function or becomes critically short) [12,13]. In a previous study, using PNA-FISH with pan-telomeric and pan-centromeric probes, we found that BLM induces short-term telomere instability in Epstein-Barr virus (EBV)--transformed human lymphoblastoid cells (produced by *in vitro* infection of human B-lymphocytic cells with EBV), in the form of incomplete chromosome elements (ICE) [5], i.e., incomplete chromosomes (chromosomes lacking one or both ends) and terminal acentric fragments (acentric fragments derived from a chromosome break at the terminal region of a chromosome, thus exhibiting telomeric signals at one end) [12,13]. In addition, our results suggested that human lymphoblastoid cells exhibit less repair capacity than human lymphocytes, with respect to BLM-induced ICE, because we found a higher frequency of these aberrations than previously observed by other authors in normal human lymphocytes exposed to this antibiotic [4]. Data on the induction of telomere dysfunction-related aberrations in human cells by BLM are very scarce. To our knowledge, the only report on the induction of telomere dysfunction-related aberrations by BLM in human cells corresponds to the one by Al-Wahiby and Slijepcevic [14]. These authors, by using the Chromosome Orientation FISH (CO-FISH) technique, showed that BLM induces chromosome-type telomere-DSB fusions but not telomere-telomere fusions in BRCA1-deficient human and mouse cell lines [14].

In order to expand our previous observations on the induction of telomere aberrations by BLM in EBV-transformed human lymphoblastoid cells, and to gain further insight into the telomere instability driven by this compound in human cells, here we present data corresponding to the short-term induction of telomere dysfunction-related chromosomal aberrations by BLM in these cells. Since telomeres play a critical role in maintaining chromosome integrity, their instability may contribute to the long-term side effects of chemotherapy related to chromosomal instability. Unlike normal human lymphocytes, EBV-transformed human lymphoblastoid cells can be continuously cultured *in vitro* for months or even years. Therefore, these cells can be used for both short- and long-term studies of mutagenesis [15–17]. Thus, EBV-transformed human lymphoblastoid cells serve as a relevant model for studying the clinical effects of BLM. This is because they enable the investigation of the long-term chromosomal instability induced by this compound, which could potentially lead to secondary cancers in patients undergoing chemotherapy based on BLM. Therefore, the use of EBV-transformed human lymphoblastoid cells could help to simulate conditions relevant to cancer patients undergoing BLM treatment that experience short- and long-term chromosomal instability. Long-term studies on the clastogenic effects of BLM on telomeres in EBV-transformed human lymphoblastoid cells are currently in progress in our laboratory.

2. Materials and methods

2.1. Cell culture, drug treatments and cell harvesting

This study was carried out using a human lymphoblastoid cell line (T-37, obtained from the IMBICE Cell Repository, La Plata, Buenos Aires, Argentina) [5,18]. This cell line was established by *in vitro* infection of human lymphocytes derived from a whole blood sample from a female donor with the Epstein-Barr virus harvested from the B95–8 marmoset cell line. Previous cytogenetic analysis in our laboratory showed that more than 90 % of T-37 cells contain 46 chromosomes [5,18], and that 24 h after BLM-treatment, $\geq$ 95 % of metaphases are in their first mitosis [18].

Cells were grown in RPMI 1640 medium (Sigma Chemical, St. Louis, MO, USA) supplemented with 20 % fetal bovine serum, penicillin (100 U/mL) and streptomycin (100 μg/mL) at 37ºC in 5 % CO_2 atmosphere. Cells were seeded in culture medium in TC25 Corning flasks at 6×10^5 cells/mL. During the log phase of growth T-37 cells were treated with a 2 h pulse of BLM (CAS no. 9041–93–4, Blocamicina®, Laboratorios Gador, Argentina, dissolved in sterile 0.9 % NaCl), 10, 25, 50 or 100 μg/mL at 37°C in order to measure concentration-dependent effects. Control cultures were not exposed to BLM. Exposure time and BLM concentrations were chosen based on previous studies carried out in our laboratory with T-37 cells exposed to this compound [5,18]. However, the highest concentration of BLM previously used in our experiments (200 μg/mL) was excluded in the present work because is highly cytotoxic for T-37 cells [5]. At the end of the pulse treatment with BLM, the cells were washed twice with Hank's balanced salt solution and kept in culture with fresh culture medium until harvesting.

To estimate cell viability, we applied the Trypan Blue exclusion assay. An aliquot of cell culture (about 30 μL) stained with 0.4 % trypan blue was used to count viable cells in a Neubauer chamber. Six independent experiments were performed to determine cell viability in control and BLM-exposed T-37 cells. The remainder of the cells were used for cytogenetic analysis, which was performed at 24 h (first mitosis) after BLM treatment. To obtain chromosome preparations, colchicine (0.1μg/mL) (Sigma, CAS No. 64–86–8) was added to cell cultures during the last 3 h of culture. After harvesting, cells were hypotonically shocked (0.075 M KCl, 30 min, 37°C), fixed in methanol: acetic acid (3:1), spread onto glass slides and stored at −20°C until processed for PNA-FISH.

2.2. Fluorescence in situ hybridization with PNA pan-telomeric and pan-centromeric probes (PNA-FISH)

A Cy3-conjugated PNA pan-telomeric probe [Cy3-$(CCCTAA)_3$] and a FAM-conjugated pan-centromeric probe obtained from PNA Bio (USA) were used. FISH was performed according to the protocol provided by the supplier in 48–72h aged slides. Briefly, after pre-treatment with 3.7 % formaldehyde and a solution containing 2 mg/mL proteinase K for 10 min, sample DNA was denatured at 85ºC for 10 min under a coverslip in the presence of the pan-telomeric and the pan-centromeric probe. Hybridization in a moist chamber (1 h at room temperature) was followed by two washes in 2X SSC 0.1 % tween 20 for 10 min at 55–60°C. Afterwards, slides were mounted on an antifade reagent containing DAPI (4,6-diamidino-2-phenylindole) as counterstain. Fluorescence microscopy was performed on a Nikon Eclipse 50i epifluorescence microscope equipped with an HBO 100 mercury lamp, a Nikon high-resolution digital color camera (DS-Ri-U3), and filters for DAPI, FAM and Cy3 (Chroma Technology Corp, Rockingham, VT).

2.3. Scoring of aberrations

Chromosome analysis was performed on coded slides, and only metaphases with 46 centromeric signals were scored. Centromeres were identified using the FAM filter, whereas telomeric signals were observed using the Cy3 filter. To obtain the final, three-color FISH images, DAPI, FAM and Cy3 images were merged using the ImageJ Software (version 1.52r). The types of telomere-dysfunction related chromosomal aberrations analyzed in the present work were telomere signal loss (TSL, chromosome- and chromatid-type), telomere signal duplication (TSD, chromosome- and chromatid-type), telomere fusion (TF), and telomere association (TA). For the identification and scoring of these aberrations we followed the criteria expressed in [13]. Besides chromosomal aberrations, we evaluated the mitotic index (MI; expressed as the percentage of cells in mitosis) by analyzing 1000 cells per sample. Five independent experiments were carried out to determine the MI in untreated and BLM-treated T-37 cells. For chromosomal aberrations analysis, two (for TSD, TF, and TA) or three (for TSL) independent experiments were performed. One hundred metaphases per sample were analyzed in the first experiment. In the second experiment, 79 (untreated control), 80 (BLM 10 and 25 μg/mL), 88 (BLM 50 μg/mL), and 93 (BLM 100 μg/mL) metaphases were analyzed, whereas 50 metaphases per sample were scored in the third experiment.

2.4. Statistical analysis of data

Statistical analysis was performed using GraphPad Prism version 9.00 software for Windows (GraphPad Software, San Diego, CA). Because different numbers of metaphases were analyzed in each experiment, the frequencies of aberrations per cell or metaphase were considered for statistical comparison of data. The significance of differences in aberration frequencies per cell among different treatments was obtained by comparing the Z score of Poisson distributions of observed (*O*) and expected (*E*) values with 95 % confidence intervals [19]. The Z score was calculated applying the following formula: $Z = O\text{-}E/\sqrt{E}$, where *O* is the total number of chromosomal aberrations observed in the corresponding treatment, and $E = P$ x N (P = frequency of chromosomal aberrations per cell or metaphase observed in the untreated culture; N = total number of metaphases analyzed in each sample or treatment). If the Z score is > 1.96, $p < 0.05$, while if Z is > 2.58, $p < 0.01$. One-way ANOVA with Dunnett's multiple comparison test was used for comparisons between untreated and BLM-treated samples in terms of cell viability and mitotix index. In both cases, the minimum level of statistical significance was set at $p < 0.05$.

3. Results

3.1. Distribution pattern of telomeric sequences in untreated cells

After telomere plus centromere PNA-FISH, centromeric signals were observed in all centromeres of the metaphase cells analyzed in the untreated controls, whereas telomeric signals of terminal position were present in most of the chromosome ends in T-37 cells. As is usually the case in EBV-transformed human lymphoblastoid cells [5,17,20–22], a few chromosomes exhibited telomere signal loss (TSL) or duplication (TSD) (Fig. 1A). Thus, some telomeres in the T-37 cell line are very short for detection by PNA-FISH. More precisely, 12 % (5059 signals) of the total number of telomeric signals expected for the 229 metaphases analyzed in the untreated cultures (42136 signals, considering a total of 184 telomeric signals per metaphase, typical of a normal human lymphocyte metaphase, i.e., the cell type from which lymphoblastoid cells are derived) were absent. Most of the telomeric signal losses (81 % on average) in untreated T-37 cells were of chromatid-type (i.e., a single signal loss per each chromosome end affected). Therefore, most of the telomeric signals (88 %) were present in the chromosomes of T-37 cells. The frequency of telomere signal duplications in untreated T-37 cells was very low (0.28 per cell, on average).

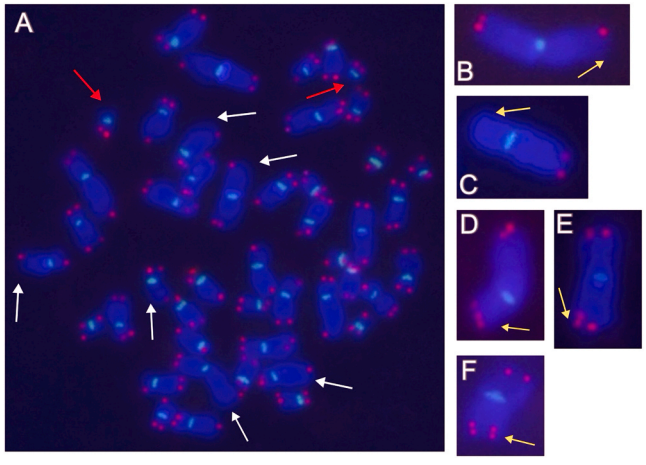

Fig. 1. (A) Patterns of hybridization of the pan-telomeric and pan-centromeric probes in a metaphase spread from untreated T-37 cells after PNA-FISH. Red/rose signals, telomeres; green signals, centromeres. Chromosomes were counterstained with DAPI (blue color). White arrows indicate sites of chromatid-type telomere signal loss (only some of them are indicated), whereas red arrows indicate sites of chromosome-type telomere signal loss. (B-D) representative examples of telomere signal loss (TSL) and telomere signal duplication (TSD) observed in BLM-exposed cells: (B) TSL, chromatid-type; (C) TSL, chromosome-type; (D-E) TSD, chromatid-type, horizontally (D) and vertically (E) oriented; (F) TSD, chromosome-type. Yellow arrows indicate the site of the aberration.

3.2. Cell viability and mitotic index

Analysis of the number of live cells (Fig. 2) and the mitotic index (Fig. 3) in each culture showed that BLM (except the concentration of 10 μg/mL) significantly decreased cell viability and mitotic index in T-37 cells 24 h after treatment, although these effects were not linear.

3.3. Telomere dysfunction-related aberrations in BLM-exposed T-37 cells

Examples of TSL and TSD observed in BLM-exposed T-37 cells are shown in Fig. 1B-F. BLM did not induce TSL in T-37 cells, whether chromatid- and chromosome-type TSL are considered together (Fig. 4, $p>0.05$) or separately (data not shown), but produced a significant concentration-dependent increase in the frequency of TSD per metaphase compared with control cultures in these cells (Fig. 5) ($p < 0.01$; Z score >2.58 for all BLM treatments compared to untreated cultures, in both experiments), although this effect was non-linear. Most of the TSD induced by BLM (95–99 %, depending on the concentration and the experiment; data not shown) were of chromatid-type, i.e., the aberration affecting only one chromatid of the chromosome end/s involved (Fig. 1D and 1E). Accordingly, a similar result was obtained when only the frequencies of chromatid-type TSD were compared between treatments (Fig. 6). However, non-significant differences were found between treatments when the frequencies of chromosome-type TSD were compared (Fig. 7). Moreover, a very low frequency of TF and TA (between 0 and 3 per treatment) was observed in BLM-exposed cells. Thus, non-significant differences were found between control culture and BLM-exposed cultures (Z score <1.96, $p > 0.05$) in terms of these

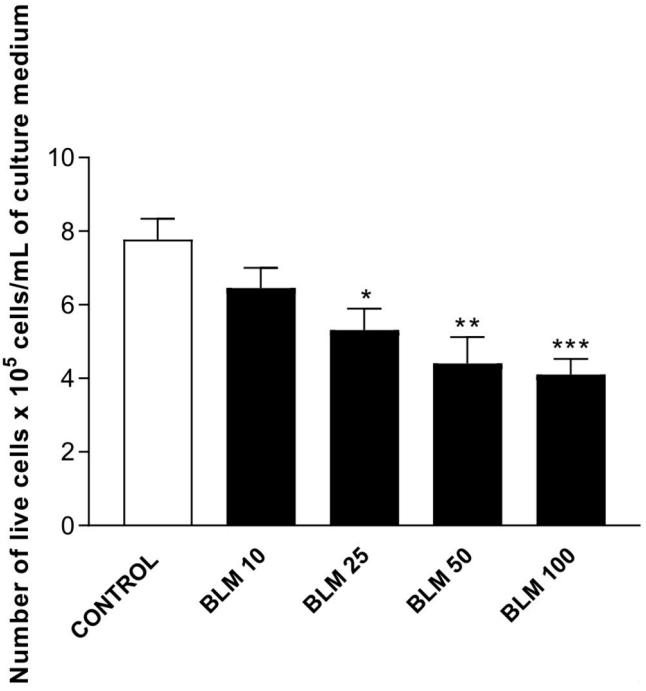

Fig. 2. Variation in the number of live cells observed in T-37 cells after treatment with increasing concentrations of BLM (μg/mL), as seen by the Trypan Blue assay. Data represent pooled values from six independent experiments. For each treatment, mean ± S.E. is indicated. (*) $p < 0.05$, (**) $p < 0.01$ and (***) $p < 0.001$ vs. untreated control culture. Statistical test: One-Way ANOVA with Dunnett's multiple comparison test.

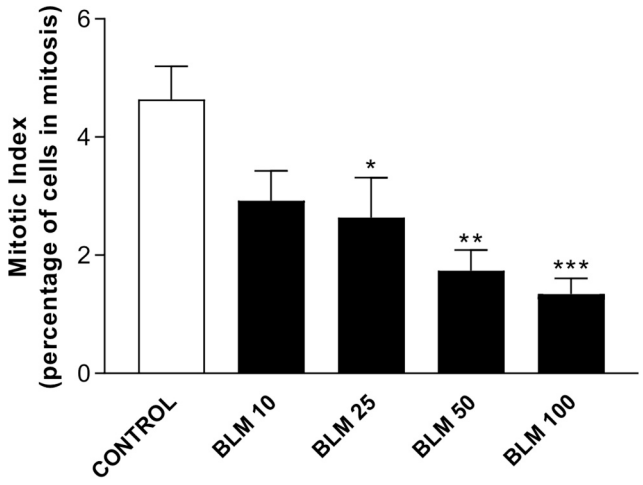

Fig. 3. Variation in the mitotic index observed in T-37 cells after treatment with increasing concentrations of BLM (μg/mL). Data represent pooled values from five independent experiments. For each treatment, mean ± S.E. is indicated. (*) $p < 0.05$, (**) $p < 0.01$ and (***) $p < 0.001$ vs. untreated control culture. Statistical test: One-Way ANOVA with Dunnett's multiple comparison test.

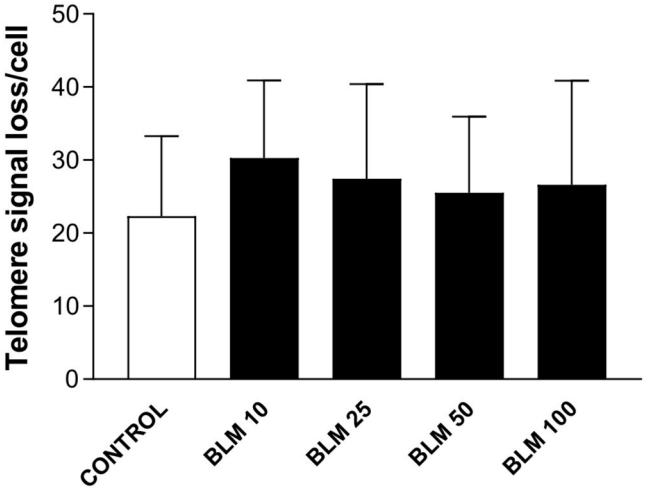

Fig. 4. Changes in the frequency of telomere signal loss (TSL) per cell in T-37 cells after treatment with increasing concentrations of BLM (μg/mL). Data represent pooled values from three independent experiments. For each treatment, mean ± S.E. is indicated. The Z score of Poisson distribution indicated non-significant differences in the frequency of TSL between untreated (control) and BLM-treated cultures ($p > 0.05$). In this graph, each TSL (no matter its type) is counted as one single event. Also, non-significant differences were found when chromosome-type and chromatid-type TSL were compared separately and when the total number of telomeric signals lost per sample was compared between treatments (data not shown).

aberrations (data not shown). No other types of telomere dysfunction-related aberrations were induced by BLM in T-37 cells.

4. Discussion

In a previous study, by using telomere plus centromere PNA-FISH, we found that BLM induces telomere instability in EBV-transformed human lymphoblastoid cells through the loss of chromosome end/s (i.e., inducing incomplete chromosome elements, giving rise to chromosomes with "open" end/s and terminal acentric fragments) [5]. In the present work, we expand these observations showing that BLM also induces telomere instability by telomere dysfunction, particularly TSD, a

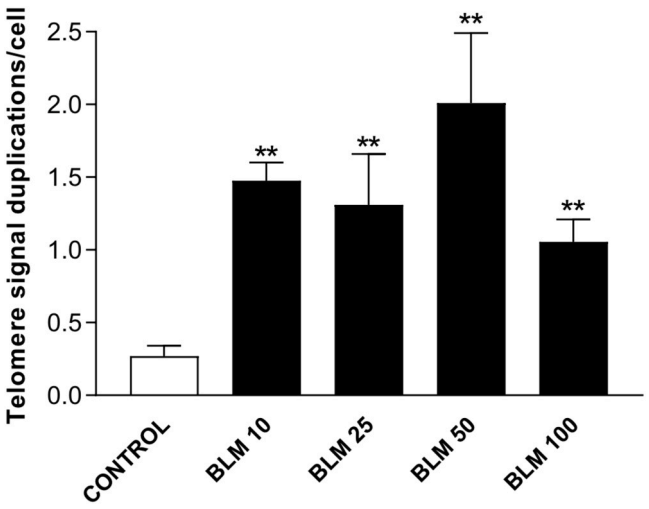

Fig. 5. Changes in the frequency of telomere signal duplications (TSD, chromosome and chromatid-type) per cell in T-37 cells after treatment with increasing concentrations of BLM (μg/mL). Data represent pooled values from two independent experiments. For each treatment, mean ± S.E. is indicated. The Z score of Poisson distribution indicated significant increase (**) in the frequency of TSD induced by BLM compared with untreated (control) cultures ($p < 0.01$). In this graph, each TSD (no matter its type) is counted as one single event. Similarly, significant differences ($p < 0.05$) were found when the total number of telomeric signals duplicated per sample was compared between treatments (in this case, each chromosome-type TSD counted as two duplicated signals; data not shown).

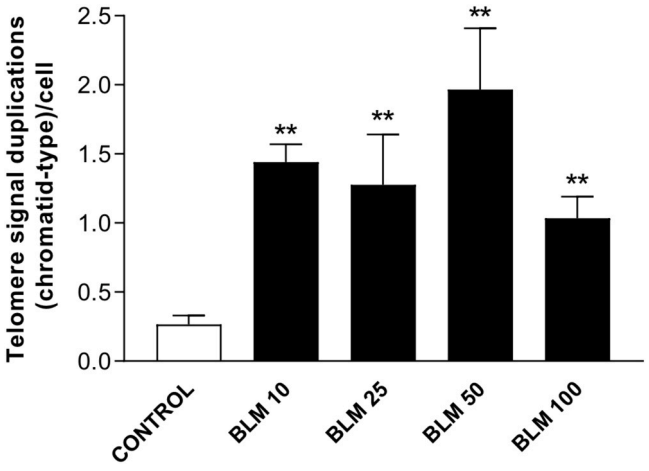

Fig. 6. Changes in the frequency of telomere signal duplications (TSD, chromatid-type) per cell in T-37 cells after treatment with increasing concentrations of BLM (μg/mL). Data represent pooled values from two independent experiments. For each treatment, mean ± S.E. is indicated. The Z score of Poisson distribution indicated significant increase (**) in the frequency of TSD induced by BLM compared with untreated (control) cultures ($p < 0.01$). In this graph, each TSD is counted as one single event.

form of telomere fragility [13]. Fragile telomeres are defined as those chromatid ends having multiple telomeric signals [13,23]. Therefore, they are cytogenetically recognized by the presence of two or more telomeric signals at one or both chromatids of one or both chromosome ends after telomere FISH [13,23]. The more basic form of fragile telomere is TSD, involving two telomeric signals resulting from the splitting of one telomeric signal into two signals. Although the molecular nature of fragile telomeres is not yet well understood, they are supposed to be the consequence of inadequate telomere DNA replication (due to the formation of single strand breaks or other lesions that stall replication

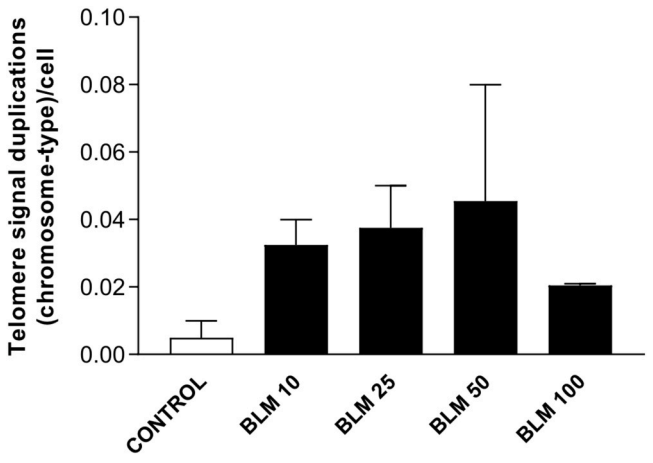

Fig. 7. Changes in the frequency of telomere signal duplications (TSD, chromosome-type) per cell in T-37 cells after treatment with increasing concentrations of BLM (μg/mL). Data represent pooled values from two independent experiments. For each treatment, mean ± S.E. is indicated. The Z score of Poisson distribution indicated non-significant increase in the frequency of TSD induced by BLM compared with untreated (control) cultures ($p > 0.05$). In this graph, each TSD is counted as one single event.

fork progression, causing an accumulation of unreplicated single stranded DNA) [24], induced by different factors (see [13] and references therein), or from break induced replication (BIR) and alt-NHEJ of the cleaved end after BIR [25]. However, because almost 100 % of TSD we found in BLM-exposed T-37 cells 24 h after treatment were of chromatid type, we may assume that telomere fragility induced by this compound in EBV-transformed human lymphoblastoid cells mainly occurs not in the S phase but in the G2 phase of the cell cycle, i.e., the telomeres of these cells are particularly sensitive to BLM in this stage of the cell cycle. The same effect was previously reported in rat cells (adipocytes) exposed to BLM 18 h and 10 days after treatment [26]. Interestingly, Ojima et al. [27] found that X-rays induce chromatid-type telomere duplication (i.e., TSD) in the long term (14 days after exposure) in normal human foreskin fibroblasts. No induction of telomere aberrations was observed in the short term (24 h after irradiation) [27]. These authors suggest that, because most telomere abnormalities observed were of chromatid-type, delayed telomere instability could occur during DNA replication or in the G2-phase. Since chromatid-type aberrations usually occur during the G2 phase of the cell cycle, we infer that BLM-induced TSD in T-37 cells is triggered at this phase. Moreover, we cannot rule out that BLM also induces TSD in the long term. Further studies are needed to confirm the above assumptions on the induction of TSD by BLM.

Fragile telomeres induced by BLM could be the result of the oxidative damage induced by this compound -as is the case with extra telomere signals induced by X-rays in mouse embryo fibroblasts [28]- and telomeric DNA fragmentation (DNA breaks), as previously reported by different authors using the Comet-FISH technique with a PNA telomeric probe, who found that BLM induces DNA breaks at telomeres in human lymphocytes, CHO cells and human cancer cell lines HT1080 and CCRF-CEM [29–31]. In fact, previous studies at the molecular level showed that BLM cleaves telomeric DNA, primarily at 5′-GT sites, and that BLM preferentially damages this type of DNA [32,33]. Therefore, our cytogenetic data are in good agreement with the ones previously obtained at the molecular level with BLM by other authors, and suggest that TSD is the cytogenetic expression of telomere DNA fragmentation induced by this compound, at least in EBV-transformed human lymphoblastoid cells.

The only previous study on telomere dysfunction-related aberrations induced by BLM refers to the induction of chromosome-type telomere-DSB but not telomere-telomere fusions in BRCA1-deficient human and mouse cell lines exposed to this antibiotic [14]. These authors used the CO-FISH technique instead of classical PNA-FISH, which allows to specifically analyze the different types of TF and also the telomere sister-chromatid exchanges (i.e., telomere recombination events) induced by a given mutagen [34]. Therefore, our study is the first one concerning the induction by BLM of telomere dysfunction-related chromosomal aberrations other than TF in human cells.

On the other hand, induction of TSL was observed in human cells 14 days after X-rays irradiation [27] and rat cells exposed to BLM 18 h after treatment [26]. In the present work, we found that BLM did not induce TSL or any other form of telomere dysfunction in T-37 cells -except TSD- in the short term. We cannot rule out the possibility that BLM can induce TSL in the long term. The only previous report of BLM-induced TSD refers to rat cells [26]. These authors found that BLM induces short- and long-term (at least 10 days after treatment) telomere instability in the form of ICE, TSL (mostly chromatid-type) and TSD (mostly chromatid-type), and also TF 10 days after treatment [26]. Recently, it was reported that X-rays do not induce TSL in mouse embryo fibroblasts in the short term [28]. Therefore, it seems that the induction of TSL by X-rays and the radiomimetic compound BLM depends both on the cell type exposed to these agents and on the time after treatment or exposure. The reasons behind these differences remain undetermined and require further investigation.

The non-linear response in the frequency of chromosomal aberrations (in our present case TSD), and in cell viability and mitotic index, induced by BLM in T-37 cells could be due to the toxicity of this compound, leading to a saturation phenomenon, clearly seen in a decreasing cell viability and mitotic index in T-37 cells exposed to this compound. In this scenario, those T-37 cells with extensive chromosome damage after BLM treatment, cannot enter mitosis. Thus, the frequency of chromosomal aberrations at high concentrations of BLM is underestimated in metaphase cells.

On the other hand, because of the occurrence of TSL in T-37 cells, we assume that the frequencies of the aberrations observed in the present work is an underestimation of the real chromosome damage induced by BLM in T-37 cells in terms of TSD. In effect, TSL could affect the detection of some TSD (i.e., there could be missing duplicated telomeres that cannot be detected), causing an underestimation of the frequencies of this type of aberration induced by BLM. However, the fact that the percentage of telomeric signals lost in BLM-treated samples (14–18 %, depending on the concentration; data not shown), was slightly higher than in the untreated samples (12 %) and that these percentages were very similar between BLM treatments, allows us to assume that TSL could equally affect the detection of TSD in all treatments. Thus, lack of some telomeric signals in control and BLM-treated samples does not invalidate the results obtained in the present work.

In summary, our study shows that BLM causes TSD -which reflect fragile telomeres- in EBV-induced human lymphoblastoid cells in the short-term. The persistence of this type of aberrations in the long-term in EBV-induced lymphoblastoid cells and other human cells exposed to BLM may be of medical relevance. Telomere fragility induced by BLM could promote genomic instability, which can be a contributing factor to secondary tumors in patients undergoing chemotherapy based on this compound. Therefore, our present study raises concerns about the potential long-term genomic effects of BLM in treated patients and suggests that the analysis of TSD could be a useful biomarker for detecting BLM-induced telomere dysfunction in human cells. Further studies are needed to elucidate if BLM induces telomere dysfunction-related chromosomal aberrations in other human cells, if this effect mainly occurs at the G2 stage of the cell cycle, and if telomere dysfunction induced by this compound in human cells persists for several generations after treatment. These experiments are currently in progress in our laboratory.

CRediT authorship contribution statement

Andrea G. Cardozo: Writing – review & editing, Methodology,

Investigation. **Daniel C. Castrogiovanni:** Writing – review & editing, Methodology, Investigation. **Julieta M. Parisi:** Writing – review & editing, Methodology, Investigation. **Alejandro D. Bolzán:** Writing – review & editing, Writing – original draft, Methodology, Investigation, Funding acquisition, Conceptualization.

Declaration of Competing Interest

The authors declare that there are no conflicts of interest.

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Data availability

Data will be made available on request.

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