Induction of glutathione-dependent DNA double-strand breaks by the novel anticancer drug brostallicin

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Abstract

Brostallicin is a DNA minor groove binder in phase II clinical trials. Here, we show that brostallicin induces γ-H2AX nuclear foci that colocalize with 53BP1 and are dependent on glutathione, as shown by inhibition of those γ-H2AX foci by L-buthionine sulfoximine. To differentiate brostallicin from the clinically approved minor groove binder trabectedin (ecteinascidin 743), we tested whether the brostallicin-induced γ-H2AX and antiproliferative responses were dependent on nucleotide excision repair and found that, unlike trabectedin, they are not. Additionally, brostallicin retained activity in the trabectedin-resistant HCT116-ER5 cell line. Induction of γ-H2AX foci by brostallicin was partially dependent on the repair nuclease Mre11. Pretreatment with aphidicolin partially reduced brostallicin-induced γ-H2AX foci, suggesting that brostallicin induces both replication-associated and replication-independent DNA damage. Replication-associated DNA damage was further shown by the colocalization of γ-H2AX foci with replication foci and by the rapid inhibition of DNA synthesis and accumulation of cells in S phase in response to brostallicin. In addition, brostallicin was able to induce lower intensity γ-H2AX foci in human circulating lymphocytes. Together, our results indicate that brostallicin induces DNA double-strand breaks and suggest γ-H2AX as a pharmacodynamic biomarker for brostallicin. [Mol Cancer Ther 2009;8(7):1985–94]

Introduction

Minor groove binders are an appealing class of anticancer agents as they possess high affinity and selectivity for interacting with DNA (see refs. 1, 2 for review). Several minor groove binders also alkylate DNA at guanine N2 or adenine N3. One such drug recently approved in Europe for the treatment of resistant or relapsed sarcomas is the marine alkaloid trabectedin [ecteinascidin 743 (Et743), Yondelis; refs. 3–5]. Although minor groove alkylating drugs generally show high antitumor activity in experimental systems, their activity is often hampered by dose-limiting bone marrow toxicity, which makes it difficult to reach therapeutic doses (1, 6–8).

The search for novel DNA alkylating agents has nevertheless continued (9, 10), and recently, a synthetic distamycin-related compound, brostallicin (PNU-166196), was selected for clinical development because of its promising activity in experimental tumor models and of its reduced bone marrow toxicity leading to an appreciable therapeutic index (2, 11, 12). In preclinical studies, brostallicin was found active against cancer cells resistant to classic DNA alkylating agents, such as cisplatin (11), and to retain activity in mismatch repair–deficient cells (13). Thus, brostallicin was proposed for patients developing resistance to alkylating agents or for combination therapies with classic alkylating agents (14, 15). Currently, brostallicin is under evaluation in phase II clinical trials with promising activity in soft tissue sarcomas (16, 17).

Brostallicin also stands apart from other DNA alkylating agents because glutathione is involved in its metabolic activation (18). Accordingly, the in vitro and in vivo activities of brostallicin are increased in tumor cells with high levels of glutathione and/or glutathione S-transferase (18). This is in contrast with classic DNA alkylating agents that tend to be inactivated by elevated glutathione, which is a common feature of cancer cells (19, 20). Thus, the overexpression of glutathione and glutathione S-transferase in tumor cells (21–25) confers a potential selectivity of brostallicin toward cancer cells and sets it apart from other DNA alkylating agents. The proposed molecular mechanism for the activation of brostallicin by glutathione involves a reaction of the α-bromoacryl moiety of brostallicin with glutathione, which is catalyzed by glutathione S-transferase, to form a highly reactive glutathione-brostallicin complex able to alkylate the exocyclic N2 position of guanines in the minor groove of DNA (Fig. 1, bottom; refs. 7, 18).

Because the induction of DNA damage by other guanine N2 minor groove alkylating agents, such as trabectedin, has recently been found readily detectable as an induction of histone γ-H2AX foci (26–28), which are landmark markers for the presence of DNA double-strand breaks (DSB; refs. 27, 29), the first aim of the present study was to determine whether brostallicin also induced γ-H2AX foci. Because we found this to be the case, the next aim of the present study was to determine the mechanism of formation of the γ-H2AX foci by testing whether those γ-H2AX foci were
dependent on glutathione and on the transcription-coupled nucleotide excision repair (NER), the latter being a characteristic of trabectedin (26, 30–32). Overall, the results from this study differentiate brostallicin from other minor groove binding agents in development.

Materials and Methods

Cells

All cell lines were maintained in DMEM containing 10% fetal bovine serum (FCS, Gemini Bio-Products). Xeroderma pigmentosum complementation group D (XP-D) fibroblasts and their stably complemented counterparts XP-D(c) were provided by Dr. Kenneth Kraemer (Basic Research Laboratory, Center for Cancer Research, National Cancer Institute, NIH, Bethesda, Maryland). Fibroblasts from a normal individual, GM00637, and xeroderma pigmentosum complementation group F (XP-F) fibroblasts were obtained from the Coriell Cell Repository (Camden, NJ). Colon carcinoma cell lines HCT116 were obtained from the Developmental Therapeutics Program (National Cancer Institute, NIH). XPG-deficient and trabectedin-resistant HCT116-ER5 cells were established in our laboratory (31). The human peripheral lymphocytes were obtained from the Blood Bank at the NIH and maintained in RPMI 1640 supplemented with 10% FCS.

Drugs and Antibodies

Brostallicin was kindly provided by Systems Medicine, a wholly owned subsidiary of Cell Therapeutics, Inc. Trabectedin (Et743) was a kind gift from PharmaMar. Stock solutions (10 mmol/L) in DMSO were stored at −20°C. Aphidicolin, l-buthionine sulfoximine (BSO), and N-acetylcycteine were purchased at Sigma Chemical Co. The anti–γ-H2AX antibody was a mouse monoclonal antibody purchased from Upstate Biotechnology. The rabbit polyclonal anti-53BP1 was purchased from Novus Biologicals.

Confocal Microscopy

Cells used for microscopy studies were grown 1 d before drug treatment in Nunc chamber slides (Nalgene). Following treatment, the medium was aspirated out and cells were washed in PBS. Cells were immediately fixed and permeabilized by a 20-min incubation at room temperature with 2% paraformaldehyde and an overnight incubation in ice-cold 70% ethanol at 4°C. After a 5-min wash in PBS, cells were incubated with 8% bovine serum albumin for 1 h at room temperature to block nonspecific binding. Cells were stained for 90 min with the primary antibodies and tagged for 45 min with fluorescent secondary antibodies (Alexa Fluor 488 or Alexa Fluor 568, 1:1,000; Molecular Probes). All incubations were made in 1% bovine serum albumin at room temperature. The primary antibodies were diluted 1:2,000 for γ-H2AX and 1:500 for 53BP1. Nuclei were stained with propidium iodide (0.05 mg/mL) and RNase A (0.5 mg/mL) for 10 min at 37°C.

For the simultaneous detection of γ-H2AX and replication foci, cells were treated with brostallicin for 2 h and labeled with 20 μmol/L 5-ethenyl-2′-deoxyuridine (EdU; Invitrogen) for the last 80 min. Following treatment, the medium was aspirated out and cells were washed in PBS. Cells were immediately fixed and permeabilized by a 20-min incubation at room temperature with 2% paraformaldehyde and an overnight incubation in ice-cold 70% ethanol at 4°C. We first did the staining for γ-H2AX as described above. The staining for EdU was then done with the Click-iT EdU flow cytometry assay kit from Invitrogen following the manufacturer’s instructions.

Slides were mounted using Vectashield mounting liquid (Vector Labs) and visualized using either a Nikon Eclipse TE-300 confocal laser scanning microscope system or a Becton Dickinson Pathway confocal microscope. Images were captured and stored as TIF files. For each sample in each experiment, 50 to 200 cells were scored. The quantification of staining intensity was done with Adobe Photoshop 7.0 and normalized to the number of cells analyzed.

Cell Viability Assays

Cells were first seeded at a density of 1,000 per well in 96-well microtiter plates. One day later, brostallicin was added and incubations were continued for an additional 72 h. Then, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) was added to each well (0.5 mg/mL; Sigma-Aldrich), and plates were maintained at 37°C for 4 h. The medium was discarded and DMSO was added to each well to lyse the cells. Absorbance was measured at 450 nm using a multiwell spectrophotometer (E_max; Molecular Devices).

Bromodeoxyuridine Incorporation and Cell Cycle Analyses

Cells were incubated with brostallicin for the indicated times and then pulse labeled with 50 μmol/L bromodeoxyuridine (BrdUr) during the last 30 min. Cells were then

Figure 1. Structure and proposed mechanism for brostallicin activation by glutathione. Adapted from Cozzi (7).
harvested by trypsinization and washed twice with PBS. The pellet containing 2 to 4 × 10^6 cells was suspended in 50 μL PBS and fixed with 1.5 mL of ice-cold 70% ethanol. After overnight storage at −20°C, cells were centrifuged and the pellet was suspended in 0.4 mL 2 N HCl. After 30-min incubation at room temperature, the medium was neutralized by the addition of 0.8 mL of 0.1 mol/L sodium borate (pH 8.5). Cells were centrifuged and the pellet was washed twice in PBS containing 0.5% Tween 20 and 0.5% bovine serum albumin. Cells were incubated with 15 μL anti-BrdUrd-FITC (BD Biosciences) for 1 h at room temperature in the dark. To determine DNA content, 500 μL of staining solution containing 20 μg/mL propidium iodide and 50 units of RNase A in PBS were added to the pellet. Cells were analyzed with a FACScan flow cytometer (BD Biosciences) using the CellQuest software (BD Biosciences).

Results

Glutathione-Dependent Induction of Histone γ-H2AX by Brostallicin

To investigate whether brostallicin induced DNA damage, we treated HCT116 cells with various concentrations of brostallicin for 6 hours and analyzed the formation of γ-H2AX foci, which is among the most sensitive methods to detect DNA damage and particularly DNA DSBs (29). Figure 2A shows that γ-H2AX foci appeared with a dose as low as 10 nmol/L brostallicin. The quantification of several experiments (Fig. 2B) shows that the γ-H2AX staining increased in a dose-dependent manner up to 1 μmol/L. The γ-H2AX response did not increase further at 10 μmol/L, consistent with other alkylating DNA minor groove agents, such as hemac, which also shows a plateau or even a decrease at high drug concentration (33). Comparable induction of γ-H2AX was observed in five other cell lines (see Fig. 4B). We also found that brostallicin was able to induce γ-H2AX in normal prostate epithelial cells (Supplementary Fig. S1).

As mentioned in Introduction, Geroni et al. (18) have shown that glutathione is necessary for the activation of brostallicin and its covalent binding to DNA (see Fig. 1). To determine whether the γ-H2AX induction by brostallicin was dependent on glutathione, we used BSO, which is an inhibitor of the γ-glutamylcysteine synthetase (one of the enzymes implicated in glutathione synthesis), to deplete intracellular glutathione (21, 34). As shown on the

1 Supplementary material for this article is available at Molecular Cancer Therapeutics Online (http://mct.aacrjournals.org/).
representative immunofluorescence confocal microscopy images (Fig. 2C) and on the quantification of several independent experiments (Fig. 2D), BSO decreased the intensity of γ-H2AX staining. This result is consistent with the fact that brostallicin-induced DNA damage is dependent on intracellular glutathione levels. The partial effect of BSO could be attributed to residual glutathione or H2AX activation by the noncovalent binding of brostallicin in the DNA minor groove.

**Brostallicin-Induced γ-H2AX Foci Persist for Several Hours after Drug Removal and Colocalize with 53BP1, Another Marker of DSB**

To further characterize brostallicin-induced DNA damage, we determined the kinetic of the appearance of γ-H2AX foci. As shown on the representative pictures (Fig. 3A) and on the quantification of the intensity of γ-H2AX staining (Fig. 3B), γ-H2AX foci were detectable after short treatments (1 hour). The intensity of the γ-H2AX staining then increased with the time of exposure in HCT116 cells. Comparable kinetics were observed in different cell lines, the NER-deficient XPD and their counterpart complemented for XPD, XPD-c (data not shown).

To further characterize the γ-H2AX foci induced by brostallicin, we did costaining experiments with both γ-H2AX and another landmark marker for DNA DSBs, 53BP1 (35). As shown on Fig. 3C, brostallicin induced the formation of 53BP1 foci that colocalized with the γ-H2AX foci. Similar induction of 53BP1 colocalizing with γ-H2AX foci was confirmed in the XPD and XPD-c cell lines (data not shown). The costaining γ-H2AX and 53BP1 is consistent with the induction of DNA DSBs by brostallicin.

Next, we investigated whether γ-H2AX foci induced by brostallicin would disappear when the drug was washed out. We treated HCT116 cells with 1 μmol/L brostallicin for 2 or 4 hours. After which, we let the cells recover in a drug-free medium for 4 and 2 hours, respectively. γ-H2AX foci were then analyzed and compared with the γ-H2AX signal measured in cells continuously incubated with brostallicin for 6 hours, which was set as 1. Columns, mean; bars, SD.
Brostallicin-Induced γ-H2AX Foci and Antiproliferative Activity Are Independent of NER, Which Sets Brostallicin Apart from Trabectedin

Like brostallicin, trabectedin (Et743), another DNA minor groove alkylating agent, is very efficient at inducing γ-H2AX foci (26–28). Figure 4A and B shows that brostallicin induced comparable levels of γ-H2AX foci as trabectedin in HCT116 cells.

Because, in the case of trabectedin, the induction of γ-H2AX is dependent on transcription-coupled NER (26, 28), we tested whether brostallicin would also induce NER-dependent γ-H2AX. To that effect, we treated three NER-deficient cell lines (XPD-deficient fibroblasts, XPC-deficient and trabectedin-resistant HCT116-ER5 cells, and XPF-deficient fibroblasts) with brostallicin and compared the induction of γ-H2AX in those cells to the induction of γ-H2AX in

Figure 4. Brostallicin and trabectedin both induce γ-H2AX foci, but neither the induction of γ-H2AX foci nor the antiproliferative activity of brostallicin depends on NER, and brostallicin-induced γ-H2AX foci are Mre11 dependent. A, comparison between brostallicin and trabectedin for the induction of γ-H2AX foci. HCT116 cells were treated for 6 h with either brostallicin or trabectedin, as indicated. γ-H2AX induction was measured by immunofluorescence confocal microscopy. Left, representative images; right, quantification of γ-H2AX staining intensity normalized to the number of cells analyzed. Columns, mean of three independent experiments; bars, SD. B, brostallicin-induced γ-H2AX foci are independent of the NER status of the cells. NER-deficient cells (XPD-, HCT116-ER5, and XPF) and their NER-proficient counterparts (XPD-c, HCT116, and GM00637, respectively) were incubated with the indicated concentrations of brostallicin for 6 h. The induction of the γ-H2AX foci was then measured by immunofluorescence. Quantification of the γ-H2AX staining intensities normalized to the number of cells analyzed. Points, mean of three independent experiments; bars, SD. For each panel, the intensity in the NER-proficient cell line incubated with 10 μmol/L brostallicin was set as 1. C, the antiproliferative activity of brostallicin is independent of NER. The same pairs used in B (NER-deficient and NER-proficient cells) were incubated with the indicated concentrations of brostallicin for 72 h and cell survival was analyzed by MTT assays. Points, mean of three independent experiments; bars, SD. D, HCT116 cells and their Mre11-complemented clone (38) were treated with the indicated brostallicin concentrations for 6 h. Left, quantification; right, representative images. The intensity of γ-H2AX staining in cells treated with brostallicin (0.1 μM in A; 1 μM in D) was set as 1.
their complemented or wild-type counterparts (the XPD-complemented XPD-c cells and HCT116 or GM00637 wild-type fibroblasts, respectively). Those cell pairs are the same we recently used to show the transcription-coupled NER-dependent induction of γ-H2AX by trabectedin (28). As shown on Fig. 4B, the induction of γ-H2AX after brostallicin treatment was similar in NER-deficient or NER-proficient cell lines, showing that, by contrast to trabectedin, brostallicin-induced γ-H2AX foci are NER independent.

In addition, we recently reported that the increased induction of γ-H2AX foci in NER-proficient cells was correlated to an increased antiproliferative activity of trabectedin (26, 28). To further investigate the lack of implication of NER in brostallicin activity, we did MTT assays in the three pairs of cell lines mentioned above (XPD versus XPD-c, HCT116-ER5 versus HCT116, and XPF versus GM00637). Figure 4C shows similar antiproliferative activity whether the cells were NER proficient or NER deficient. Altogether, the γ-H2AX and the cell proliferation assays show that brostallicin acts independently of the NER and thus show that brostallicin is different than trabectedin. Additionally, brostallicin still retained activity in the

Figure 5. Brostallicin-induced γ-H2AX foci are mostly replication dependent. A, γ-H2AX inhibition by the DNA polymerase inhibitor aphidicolin (APD). HCT116 cells were treated with brostallicin in the presence or absence of aphidicolin, as indicated. Left, representative pictures; right, quantification of γ-H2AX staining intensity normalized to the number of cells analyzed. Columns, mean of three independent experiments; bars, SD. The intensity of the γ-H2AX staining in HCT116 cells treated with brostallicin alone was set as 1. B, brostallicin-induced γ-H2AX foci colocalize with replication foci visualized by EdU incorporation. HCT116 cells were treated with 1 μmol/L brostallicin for 2 h and pulse labeled with 20 μmol/L EdU. Top, γ-H2AX staining; bottom, EdU staining. Insets, arrowheads, cells with γ-H2AX and EdU colocalization; asterisks, γ-H2AX-positive cells without significant EdU staining. C, brostallicin-induced γ-H2AX foci in human lymphocytes. D, quantification of γ-H2AX staining intensity normalized to the number of cells analyzed.
HCT116-ER5 cell line generated in our laboratory for trabectedin resistance (31). These findings also suggest that cross-resistance between trabectedin and brostallicin may not occur.

**Mre11 Increases the Brostallicin-Induced γ-H2AX Response**

Mre11 is a DNA repair endonuclease, which has been associated with DSB repair (36). To determine whether Mre11 was involved in brostallicin-induced DSBs, we compared the induction of γ-H2AX in Mre11-deficient HCT116 cells (37, 38) and in their Mre11-complemented clone (38). Figure 4D shows enhanced induction of γ-H2AX in Mre11-complemented cells, which indicates that the γ-H2AX response to brostallicin is partially dependent on Mre11 activity.

**Replication Dependence of the Brostallicin-Induced γ-H2AX Foci**

Examination of the γ-H2AX responses of different cells simultaneously treated in a given experiment with brostallicin showed that not all cells responded similarly. Some cells exhibited intense response with multiple foci, whereas some had only a few foci and some others showed minimal γ-H2AX foci (see Figs. 2–4). Our prior studies revealed that such differential staining could be attributed to replication-dependent γ-H2AX induction in response to replication-associated DNA damage (39, 40). To investigate whether brostallicin-induced DNA damage was related to replication, we treated cells with aphidicolin, a DNA polymerase inhibitor, which we previously used to prevent γ-H2AX induction by replication-associated DNA damage (39, 40). Figure 5A shows that aphidicolin reduced brostallicin-induced γ-H2AX, which is consistent with replication-associated DNA damage.

Because the suppressive effect of aphidicolin on brostallicin-induced γ-H2AX was only partial (Fig. 5A) and some cells without significant EdU labeling still showed γ-H2AX foci (Fig. 5B, inset, asterisks), we investigated whether brostallicin could also induce γ-H2AX in nonreplicating cells. Figure 5C and D shows that brostallicin could induce γ-H2AX foci in normal human lymphocytes, albeit with higher doses of brostallicin. Those results show that, at low dose, the γ-H2AX induction by brostallicin was mostly dependent on replication, whereas micromolar concentrations of brostallicin were able to induce γ-H2AX independently of replication.
Inhibition of DNA Replication and S-Phase Synchronization by Brostallicin

As we found that brostallicin induced replication-associated DNA damage, we tested the effect of brostallicin on cell cycle progression and DNA synthesis. Flow cytometry analyses (Fig. 6A) showed that brostallicin induced a marked accumulation of the cells in the S phase of the cell cycle after 24-hour drug exposure. The G1 and G2-M phases were almost completely depleted. The percentage of cells in G1 dropped from 44.0 ± 2.5% in untreated cells to 4.9 ± 2.0% in brostallicin-treated cells and the percentage of cells in G2-M dropped from 14.3 ± 2.1% in untreated cells to 2.0 ± 2.0% in brostallicin-treated cells. Almost all the cells were distributed in S phase (from 41.8 ± 1.5% in control cells to 93.1 ± 0.1% in brostallicin-treated cells; Fig. 6A).

In parallel experiments, we measured DNA synthesis in brostallicin-treated cells by BrdUrd pulse incorporation. As shown on the representative fluorescence-activated cell sorting plots and by quantifying independent experiments (Fig. 6B), brostallicin reduced BrdUrd incorporation, with BrdUrd-positive cells dropping from 34.8 ± 3.2% BrdUrd-positive cells in control cells to 16.8 ± 2.3% and 13.0 ± 2.7% in cells treated with brostallicin for 4 and 6 hours, respectively. These results are consistent with inhibition of DNA synthesis by brostallicin.

Discussion

In this study, we have shown that brostallicin induces DNA damage, particularly DNA DSBs, as revealed by the induction of γ-H2AX and 53BP1 foci (Figs. 2 and 3). Consistently with the alkylation of DNA by brostallicin, we show that washing out the drug did not decrease the induction of γ-H2AX and that the induction of DNA damage on brostallicin treatment was persistent (Fig. 2). Also in accordance with the proposed glutathione-dependent DNA alkylation by brostallicin (Fig. 1; refs. 7, 18), we found that the induction of γ-H2AX was reduced when the intracellular levels of glutathione were depleted by BSO. Together, the above results further justify the rationale for using brostallicin in tumors with high glutathione levels, which is a relatively common feature of cancer cells especially after prior chemotherapy (7, 18-20, 23-25, 44).

The inhibition of brostallicin-induced γ-H2AX in cells treated with the DNA polymerase inhibitor aphidicolin (45) and the colocalization of those γ-H2AX foci with replication factories detected by the incorporation of an analogue of thymidine, EdU (Figs. 5 and 6), show replication-dependent induction of γ-H2AX by brostallicin. The fact that brostallicin inhibited DNA synthesis and led to an accumulation of brostallicin-treated cells in S phase (Fig. 6) is also consistent with a prominent effect of brostallicin on DNA replication. Replication-coupled DSBs are well known for other anticancer agents, such as the camptothecins (38, 39). In that case, DNA polymerase runoff converts the DNA single-strand breaks generated by the trapping of topoisomerase I-DNA complexes into DSB (46). We have recently reported an alternate mechanism with another novel anticancer agent, aminoflavone derivative (NSC 686288), which produces DNA-protein complexes that arrest replication fork progression (40) and may involve “replication fork collapse” (47). Abnormal DNA structures and helicase deficiencies have also been shown to produce replication-coupled DSB that can be detected as γ-H2AX foci (48). Replication-coupled DSBs have also been reported for DNA alkylating agents, which like brostallicin alkylate guanine N2 in the DNA minor groove. Indeed, both trabectedin and S23906 (9, 27, 28) generate γ-H2AX foci that were proposed to occur when an advancing DNA replication fork runs into a DNA adduct. Both models (collision of the replication fork either with a DNA-protein-drug complex or a DNA adduct) are consistent with the decrease of γ-H2AX induction produced by aphidicolin and the colocalization of γ-H2AX foci with replication factories (see Fig. 5; ref. 41).

In addition to replication-mediated γ-H2AX foci, we show here that brostallicin can induce γ-H2AX independently of replication based on the finding that aphidicolin only partially reduces the γ-H2AX response (see Fig. 4A), based on the presence of γ-H2AX foci in cells outside of S phase, and based on the H2AX response of postmitotic peripheral lymphocytes at micromolar concentrations of brostallicin (see Fig. 4B). Replication-independent DSBs have also been reported for trabectedin (28) and the pluramycin derivative hedamycin (33). Whether such DSBs are related to transcription, as in the case of trabectedin (28), remains to be elucidated.

Because brostallicin and trabectedin are both DNA minor groove alkylators and are clinically active in the same types of tumors (soft tissue sarcomas; refs. 4, 16), we compared the molecular and cellular effects of the two drugs. We find that trabectedin and brostallicin do not have the same dependency on NER neither in terms of antiproliferative activity nor in terms of induction of γ-H2AX foci. γ-H2AX foci induction on brostallicin treatment was the same in cells proficient or deficient for NER (Fig. 4B and C). Accordingly, NER-deficient cells did not exhibit differential sensitivity to brostallicin (Fig. 4C). We also show that brostallicin retains full activity in the HCT116-ER5 cells (Fig. 4C; ref. 31), which further differentiates brostallicin from trabectedin. Thus, our study shows key mechanistic differences between brostallicin and trabectedin with respect to the molecular and mechanistic determinants of γ-H2AX induction (primarily replicative in the case of brostallicin and NER dependent in the case of trabectedin) and NER dependency. By contrast, although the detailed molecular mechanisms leading to the formation of DSBs remain to be fully determined, we found that the DSB-associated nucleosome Mre11 that increased the γ-H2AX induction by brostallicin was partially dependent on the DSB-associated nucleosome Mre11 (see Fig. 4D). We also recently reported that Mre11 was involved in the induction of γ-H2AX by trabectedin (28), which indicates that both alkylation agents are able to induce DSBs in connection with Mre11.

In conclusion, our study establishes some unique characteristics that set brostallicin apart from presently used anticancer agents. Indeed, the DNA damaging activity of
brostallicin is enhanced by high levels of glutathione, which is a hallmark of drug-resistant tumor cells (7, 18, 21–25). We also show for the first time that brostallicin selectively damages and blocks replicating DNA within replication factories. These results, with the detection of γ-H2AX foci in peripheral lymphocytes on brostallicin treatment, suggest γ-H2AX detection as a useful pharmacodynamic biomarker (28, 29, 49) in the upcoming clinical trials with brostallicin. The rapid appearance and persistence of γ-H2AX for several hours following brostallicin removal make γ-H2AX a potentially useful pharmacodynamic marker for the ongoing brostallicin clinical trials.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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References

32. Zewail-Foote M, Li VS, Kohn H, Bears D, Guzman M, Hurley LH. The inefficiency of incisions of ecteinascidin 743-DNA adducts by the UV-ABC nuclease and the unique structural feature of the DNA adducts can be used to explain the repair-dependent toxicities of this antitumor agent. Chem Biol 2001;8:1033–49.


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