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An unusual DNA binding compound, S23906, induces mitotic catastrophe in cultured human cells

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ABSTRACT

The biochemical pathways that lead cells to mitotic catastrophe are not well understood. To identify these pathways, we have taken an approach of treating cells with a novel genotoxic compound and characterizing whether cells enter mitotic catastrophe or not. S23906 is a novel acronycine derivative that forms adducts with the N2 residue of guanine in the minor groove of the DNA helix and destabilizes base pairing to cause helix opening. We observed, in HeLa and HT-29 cells, that S23906 induced γ-H2AX and activated checkpoint kinase 1, as did bleomycin, camptothecin, and cisplatin, when tested under equi-toxic conditions. S23906 also induced cyclin E1 protein, although this activity was not required for cytotoxicity because knock down of cyclin E1 by RNA interference did not affect the number of dead cells after treatment. Cyclin B1 levels first decreased and then increased after treatment with S23906. Cyclin B1 was associated with Cdk1 kinase activity, which correlated with an increase in the number of mitotic cells. By 32 h after treatment, at least 20% of the cells entered mitotic catastrophe as determined by microscopy. Suppression of the DNA checkpoint response by co-treatment with caffeine increased the number of cells in mitosis. These results suggest that mitotic catastrophe is one of the cellular responses to S23906 and that mitotic catastrophe may be a common cellular response to many different types of DNA damage.

1. Introduction

The majority of cytotoxic cancer treatments, such as ionizing radiation, topotecan, platinum derivatives and alkylating agents damage DNA. At the cellular level, these treatments initiate a series of biochemical events that lead to a DNA damage checkpoint. Once damaged DNA is detected by the cell, the protein kinases ATM (Ataxia telangiectasia mutated) and ATR (Ataxia telangiectasia mutated-RAD3 related) phosphorylate histone H2AX [1,2] and checkpoint kinases (Chk1 and Chk2) [3,4]. Chk1 then phosphorylates members of the Cdc25 phosphatase family, which leads to their inactivation by sequestration [5,6] or by degradation [7]. Without Cdc25 phosphatases, cyclin-dependent protein kinase 1 (Cdk1) remains inactive and causes cell cycle arrest. This arrest prevents cells from entering mitosis and protects daughter cells from receiving damaged DNA. The biochemical steps of the DNA damage checkpoint can be followed by detection of the key members of the pathway, such as increased expression of histone γ-H2AX, phosphorylated Chk1, and low levels of Cdk1 activity.

Abbreviations: CPT, camptothecin; CDDP, cisplatin; Cdk1, cyclin-dependent kinase 1; Chk1, checkpoint kinase 1.

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The term mitotic catastrophe was first used to describe a failed mitosis in *Schizosaccharomyces pombe* mutants in which wee1, the negative regulator of cdc2, was mutated causing cells to enter mitosis with incompletely replicated DNA [8]. The term also applies to cells that are unsuccessfully segregating damaged DNA [9]. Mitotic catastrophe is identified by observation of poorly formed mitotic chromosomes and micronuclei typically in cells that have been treated with genotoxic agents [9,10]. Biochemical evidence of mitotic catastrophe includes loss of Chk1 function and increase in Cdk1 kinase activity [11,12].

The compound S23906 is an atypical alkylating agent that forms monoadducts on the N2 of guanine in the minor groove of DNA. In tests using HT-29 cell lines, S23906 inhibits DNA synthesis, increases cyclin E1 protein levels and causes cell death [13]. Further analysis in KB-3-1 cells and a resistant cell line revealed that S23906 induces histone γ-H2AX and checkpoint kinase 2 activation in a dose dependent manner [14]. These results show that S23906 induces DNA damage, which is responsible for its cytotoxic effect on a large variety of cell lines and in tumor models in mice [15].

In this report, we examine if S23906 induces Chk1 activation followed by mitotic catastrophe. Mitotic catastrophe is a frequent outcome to genotoxic treatments such as irradiation [16] or the radiomimetic bleomycin [17], by inhibition of topoisomerase I by camptothecin [18,19], and by DNA crosslinking after treatment with cisplatin [20,21]. Whether all types of DNA damage lead to mitotic catastrophe, or whether it is a response to subclass of genotoxic agents is not known.

We reasoned that if S23906 causes mitotic catastrophe, with its unusual mechanism of damaging DNA, it could be taken as evidence that mitotic catastrophe is a common response to many, if not all, types of DNA damage. Therefore, we examined the cellular response of human cancer cell lines to the S23906 by characterizing the components of the DNA damage signaling pathway and by measuring the levels of proteins required in mitosis. We found that cells treated with pharmacological concentrations of S23906 engage a checkpoint response and enter mitotic catastrophe.

2. Materials and methods

The human cell lines HT-29 and HeLa were obtained from the American Type Culture Collection. HT-29 cells were maintained in RPMI 1640 medium supplemented (RPMIc) with 10% decomplemented fetal calf serum (Sigma), 2 mM L-glutamine (Invitrogen), and 10 mM HEPES, pH 7.4. Cells were grown at 37 °C in 5% CO_2. HeLa cells were maintained in DMEM with 10% decomplemented fetal calf serum (Sigma), 2 mM L-glutamine (Invitrogen), and 10 mM HEPES, pH 7.4. The compounds camptothecin (CPT, Sigma), cisplatin (Bellon-Aventis, France), S23906 (Servier) were dissolved in DMSO to a concentration of 10 mM and stored at −20 °C until use. Bleomycin (Sigma) was stored at 400 mU/mL at −20 °C. The compounds were used at the following final concentrations camptothecin (CPT) 100 nM, bleomycin 40 mUnits/mL, cisplatin (CDDP) 15 μM and S23906 at 1 μM unless stated otherwise. To suppress the DNA damage checkpoint,

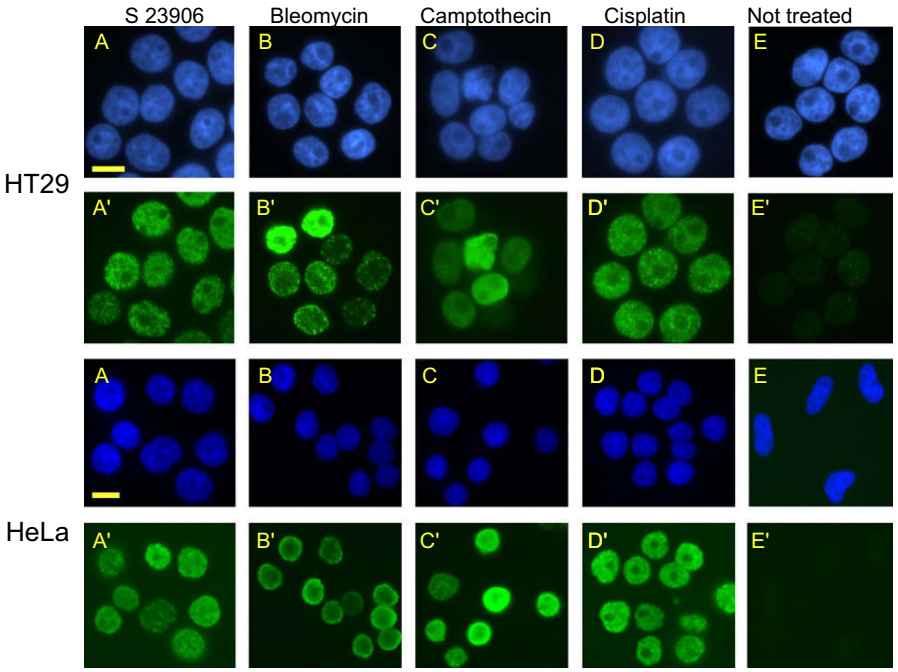

Fig. 1. DNA damage induced in two cell lines after treatment with S23906. HT-29 cells and HeLa cells were treated with equi-toxic concentrations of S23906 (1 μM), or bleomycin (40 mU/mL), or camptothecin (100 nM), or cisplatin (15 μM) for 24 h. Cells were then examined by immunofluorescence microscopy to detect nuclei by DAPI staining (top rows A–E) or histone γ-H2AX staining (bottom rows A′–E′). Control cells that did not receive any treatment are shown for comparison (not treated). Bars represent 10 μm.