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Proteasomal inhibition stabilizes topoisomerase IIα protein and reverses resistance to the topoisomerase II poison ethonafide (AMP-53, 6-ethoxyazonafide)

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ABSTRACT

Multiple myeloma (MM) is an incurable malignancy of plasma cells. Although multiple myeloma patients often respond to initial therapy, the majority of patients will relapse with disease that is refractory to further drug treatment. Thus, new therapeutic strategies are needed. One common mechanism of acquired drug resistance involves a reduction in the expression or function of the drug target. We hypothesized that the cytotoxic activity of topoisomerase II (topo II) poisons could be enhanced, and drug resistance overcome, by increasing the expression and activity of the drug target, topo II in myeloma cells. To test this hypothesis, we evaluated the cytotoxicity of the anthracene-containing topo II poison, ethonafide (AMP-53/6-ethoxyazonafide), in combination with the proteasome inhibitor bortezomib (PS-341/Velcade). Combination drug activity studies were done in 8226/S myeloma cells and its drug resistant subclone, 8226/Dox1V. We found that a 24-h treatment of cells with bortezomib maximally increased topo IIα protein expression and activity, and consistently increased the cytotoxicity of ethonafide in the 8226/S and 8226/Dox1V cell lines. This increase in cytotoxicity corresponded to an increase in DNA double-strand breaks, as measured by the neutral comet assay. Therefore, increasing topo IIα expression through inhibition of proteasomal degradation increased DNA double-strand breaks and enhanced the cytotoxicity of the topo II poison ethonafide. These data suggest that bortezomib-mediated stabilization of topo IIα expression may potentiate the cytotoxic activity of topo II poisons and thereby, provide a strategy to circumvent drug resistance.

1. Introduction

Multiple myeloma is a B-cell malignancy characterized by the accumulation of latent plasma cells in the bone marrow. Patients with multiple myeloma frequently respond to initial drug therapy, but later relapse with disease that is refractory to further treatment. In spite of recent advances in molecular therapeutics, the disease is uniformly fatal, and the median survival remains 36–60 months, with no significant improvements in long-term survival in the past 20 years [1].

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One class of chemotherapeutic agents classically used to treat patients with relapsed myeloma is drugs that inhibit topoisomerase II (topo II), including doxorubicin, etoposide, and mitoxantrone. Topo II is an ATP-dependent enzyme that catalyzes changes in DNA topology necessary for transcription, replication, and chromosome condensation and segregation [2,3]. Because topo II is essential for DNA replication, it has been identified as an important drug target in the treatment of cancer. Topo II exists in two isoforms: topo IIα is cell-cycle regulated and is necessary for chromosome condensation and segregation in mitosis, whereas topo IIβ is constitutively expressed and has been shown to be involved in DNA repair and transcription [4,5]. When administered as part of a combination chemotherapy regimen, topo II poisons contribute to the improved rates of remission and survival for many hematological malignancies including childhood acute lymphocytic leukemia (ALL), adult acute myelogenous leukemia (AML) and multiple myeloma [3]. Nevertheless, relapse associated with acquired resistance to topo II poisons remains a clinical challenge.

Known mechanisms of acquired drug resistance to topo II poisons include both modification of drug transport and alteration of the drug target. For example, the over-expression in tumor cells of the efflux transporter P-glycoprotein, encoded by the *mdr1* gene, can result in resistance to a wide range of anticancer drugs that vary structurally and functionally. This phenomenon is known as the multidrug resistance (MDR) phenotype [6,7].

Although resistance to topo II poisons is often observed in tumor cells that over-express P-glycoprotein, the frequency of clinical resistance cannot be explained by P-glycoprotein-mediated efflux alone. Reduction in topo II protein levels and activity have been proposed to be potentially more important mechanisms of resistance to topo II poisons [7]. Previously published studies of various cell lines that have been selected for resistance to topo II poisons indicates a variety of potential mechanisms leading to reduced topo II expression and activity. Topo II activity can be modulated by a decrease in expression of the gene due to either reduced transcription or translation, an alteration of the coding sequence leading to the production of an enzyme with modified activity, or post-translational modifications of the enzyme. These activities all may result in the observed phenotype of a reduction in topo II expression and activity [8].

Previous studies have investigated the emergence of the drug resistant-phenotype in the human multiple myeloma cell line RPMI 8226 (8226/S). When cells were selected for doxorubicin resistance (8226/Dox40), drug resistance was mediated by P-glycoprotein over-expression [9]. In contrast, when cells were selected for resistance to doxorubicin in the presence of the P-glycoprotein inhibitor, verapamil (8226/Dox1V), drug resistance was associated with reduced expression and activity of topoisomerase IIα with no induction of P-glycoprotein over-expression [10]. In the present study, the 8226/Dox1V cell line was used as a model to investigate potential strategies to reverse resistance to topo II poisons associated with a reduction in topo IIα expression and activity.

Topoisomerase II is an ATP-dependent enzyme that catalyzes changes in DNA topology by passing an intact double helix through a transient double-stranded DNA break. A critical step in the reaction catalyzed by topo II involves the formation of a topo II-DNA covalent complex, referred to as the cleavable complex, in which each topo II homodimeric subunit is covalently linked to the 5′-phosphoryl ends of the broken DNA strand [11,12]. Under normal circumstances, the cleavable complex is a short-lived reaction intermediate. However, a persistence or stabilization of cleavable complexes leads to an accumulation of DSBs in the genome of the cell and therefore has cytotoxic effects [11]. Thus, anticancer activity of topo II poisons is directly associated with stabilization of the cleavable complex and resulting DNA strand breaks.

Previous studies have shown that the cell cycle-dependent expression of topo IIα is regulated by proteasomal degradation [13]. The 26S proteasome is a multicatalytic enzyme complex that is the primary component of the protein degradation pathway of the cell [14–16]. Inhibition of the proteasome is therefore a promising approach for cancer treatment. Bortezomib (PS-341/Velcade) is a dipeptide boronic acid inhibitor that is highly selective for the proteasome, having little affinity for other proteases (Fig. 1A). Bortezomib forms a covalent bond with the active site threonine in the core of the 20S proteasome, and inhibits the chymotryptic activity of the proteasome [17]. In 2003, bortezomib was approved in the

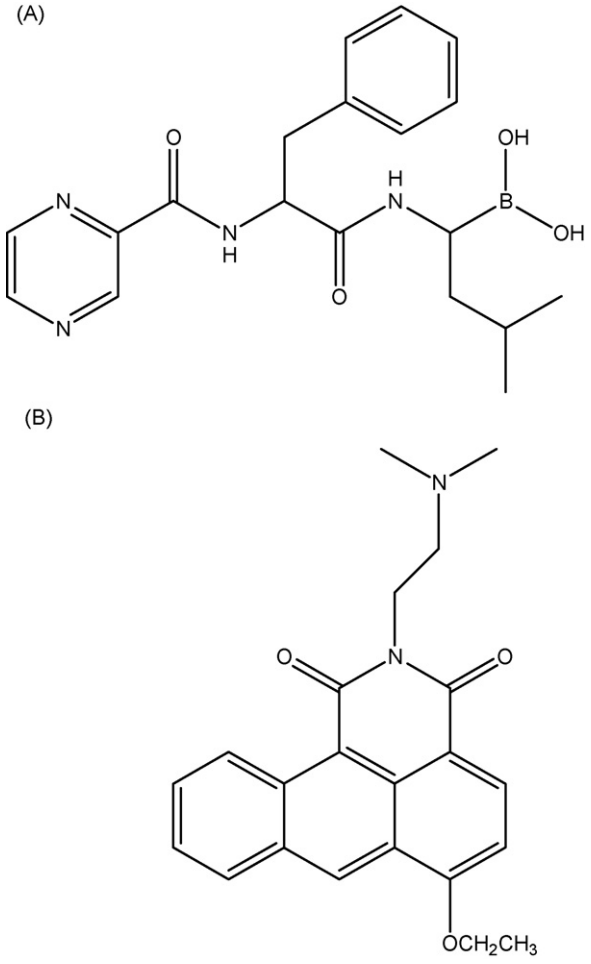

Fig. 1 – (A) Chemical structure of bortezomib (PS-341/Velcade). (B) Chemical structure of ethonafide (AMP-53/6-ethoxyazonafide).