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Epothilone-paclitaxel resistant leukemic cells CEM/dEpoB300 are sensitive to albendazole: Involvement of apoptotic pathways

Azita Khalilzadeh, Kiran T. Wangoo, David L. Morris, Mohammad H. Pourgholami *

Cancer Research Laboratories, University of New South Wales, Department of Surgery, St. George Hospital, Sydney, NSW 2217, Australia

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ABSTRACT

Altered or deficient activation of apoptosis signalling pathways may contribute to drug resistance. Here, we assess the role of apoptotic mediators in eliciting an anti-proliferative response to paclitaxel (PTX) in a T cell acute lymphoblastic leukemia (ALL) cell line CEM and its epothilone-paclitaxel resistant sub-line CEM/dEpoB300. Furthermore, the cellular response to PTX was compared to those elicited by cells in response to treatment with albendazole (ABZ; a microtubule depolymerizing agent). In cell proliferation studies, CEM cells were sensitive to both PTX and ABZ, while the CEM/dEpoB300 cells were highly resistant to PTX (IC_{50} 2.86 nM versus 30.26 nM, respectively). In contrast, the resistant cells showed a 2-fold increase in sensitivity to ABZ (0.32 μM in CEM compared to 0.16 μM in CEM/dEpoB300). Analysis of caspase-3 activity and cytochrome *c* release in response to PTX or ABZ treatment (24, 48 and 72 h) revealed that, compared to the parent cells, the resistant cells have diminished response to PTX and enhanced response to ABZ. A similar pattern was observed for the pro-apoptotic protein Bax. Levels of the anti-apoptotic protein Bcl-2 was highly elevated in CEM/dEpoB300 cells and in these cells, ABZ was more effective in lowering the Bcl-2 levels than PTX. Similarly, ABZ treatment led to profound down regulation of the Mcl-1 protein. These results reveal for the first time, the changes in apoptotic mediators following development of resistance to PTX in an ALL cell and the significantly increased sensitivity of these PTX resistant cells to ABZ.

1. Introduction

Acute lymphoblastic leukaemia (ALL) is the most frequent childhood cancer. Whereas the majority of patients will be long-term survivors, almost one-third of patients will relapse and die because of the development of drug resistance [1]. Natural product antimitotic drugs targeting the tubulin/microtubule system are important in the treatment of a range of hematologic and solid cancers. One of these agents, PTX, is used in the treatment of a variety of cancers including lung, ovarian, breast cancer and leukaemia [2,3]. The success of these drugs is related to their mechanism of action that leads to disruption of cell division and induction of apoptosis [4]. However, the clinical usefulness of these agents is hampered by high levels of neurological and bone marrow toxicity and the emergence of drug-resistant tumour cells [4]. To overcome these problems and to improve therapy, investigations have focused on identifying and developing new agents that target

* *Corresponding author at*: Department of Surgery, St. George Hospital, Kogarah, Sydney, NSW 2217, Australia. Tel.: +61 2 9350 2975; fax: +61 2 9350 3997.
E-mail address: mh.pourgholami@unsw.edu.au (M.H. Pourgholami).
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tubulin. Epothilones, a new group of β-tubulin acting agents, have been developed with the aim of treating PTX resistant tumours [5,6]. While still not in clinical use, experimental *in vitro* studies have shown development of resistance to epothilones by the leukemic cells CEM. Interestingly, the resultant sub-line (CEM/dEpoB300) was both epothilone and PTX resistant [2].

Albendazole, a widely used broad spectrum benzimidazole carbamate (BZD) anthelmintic drug with an excellent safety record [7] has been shown to bind to β-tubulin and act as a depolymerizing agent [8,9]. In recent years, we have reported potent anti-tumour and more recently potent anti-VEGF properties for ABZ [10,11]. We therefore hypothesised that because of the opposing effects on the microtubule, ABZ may display anti-proliferative effects in CEMdEpoB300 PTX resistant tumour cells.

To examine this hypothesis, a comparative study was designed to look at the effects of PTX and ABZ on the leukemic cells CEM and the PTX resistant sub-line CEM/dEpoB300 in terms of both cytotoxicity and the apoptotic mediators involved.

Results obtained not only provide novel insights into the involvement of anti-apoptotic mediation of resistance to PTX but also reveal for the first time how in PTX resistant cells, ABZ changes the levels of pro-apoptotic to anti-apoptotic mediators favouring apoptosis and hence leading to enhanced anti-proliferative effects. Interestingly, a very recent report confirms the clinical importance of these mediators in determining therapeutic response to chemotherapy [12].

2. Materials and methods

2.1. Cell culture

Human T-cell acute lymphoblastic leukemia cells, CEM, and its desoxyepothilone B resistant sub-line, CEM/dEpoB300 were kindly provided by Dr. Maria Kavallaris of Children's Cancer Institute Australia for Medical Research. Cells maintained in RPMI-1640 medium supplemented with 10% fetal bovine serum and were grown in a humidified incubator at 37 °C under a 5% CO_2/95% air atmosphere. For all experiments, 5×10^5 cells/ml were incubated with or without drugs for 24, 48 or 72 h.

2.2. Cell proliferation assay

Anti-proliferative effects of PTX and ABZ on CEM, and CEM/dEpoB300 was determined by the MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide) assay [13]. Cells seeded in 96-well plates (15,000 cell/well) were treated with various concentrations of PTX (1–10,000 nM) or ABZ (0.01–10 μM). After 72 h of incubation 0.5 mg/ml of MTT was added to each well and plates were incubated for a further 4 h for colour development. Following this, cells were solubilized by the addition of 100 μl of dimethyl sulfoxide to each well. The absorbance was read at 562 nm on an automated plate reader. Each drug concentration was tested in a replicate of 8 and each experiment was performed at least twice and results are presented as the mean ± standard error (S.E.M.).

2.3. Preparation of cytosolic extract and measurement of caspase-3 activity

CEM and CEM/dEpoB300 cells seeded at density of 5×10^5 cells/ml were incubated for 24, 48 and 72 h with 10 nM PTX or 0.25 μM ABZ. Drug concentrations were chosen on the basis of PTX and ABZ efficacy from cell proliferation assays. Cells were then separated from the medium by centrifugation at 1500 × *g* for 5 min at 4 °C and washed twice with phosphate-buffered saline (PBS). Caspase-3 levels in vehicle and drug treated samples were measured by a kit according to the procedures provided by the manufacturer (Caspase Colorimetric Assay kits; R&D Systems, Minneapolis, USA). The cell pellets were resuspended in lysis buffer and left on ice for 10 min. The lysates were centrifuged at 10,000 × *g* for 1 min and the supernatant (50 μl) was used to evaluate the caspase-3 level. The plate reader was calibrated using standard samples of caspase-3 and the absorbance was measured at 405 nm and used as being indicative of caspase-3 activity.

2.4. Determination of cytochrome c release

Cells were seeded and treated as described above. The cytosolic fraction obtained from cells (10^6) suspended in 100 μl of cold buffer (80 mM KCl and 200 μg/ml digitonin in PBS) was centrifuged (800 × *g*) for 5 min. The supernatant was used to determine cytochrome *c* levels (Cytochrome *c* Immunoassay Kit; R&D Systems, Minneapolis, USA). Following completion of procedures, the absorbance was measured at 450 nm.

2.5. Measurement of Bcl-2 and Bax

CEM and CEM/dEpoB300 treated up to 72 h with either PTX (10 nM) or ABZ (0.25 μM), were harvested as described above for cytochrome *c* and the level of anti-apoptotic Bcl-2 protein and pro-apoptotic protein Bax available in each sample was measured using standard enzyme immunoassay kit (Human Bcl-2 and Human Bax Enzyme Immunometric Assay Kits; Assay Designs, Sydney, Australia). Briefly cells were washed with PBS and lysed with 1 ml of Bcl-2 or Bax lysis buffer (provided by the manufacturer) for 5 and 15 min, respectively, at 4 °C. Cell lysates were centrifuged at 16,000 rpm for 15 min and the supernatant were used to determine levels of each protein.

2.6. Bcl-2 and Bax mRNA determination

CEM and CEM/dEpoB300 cells treated with vehicle, PTX or ABZ were harvested and examined for the expression of Bcl-2 and Bax by real time quantitative RT-PCR (qRT-PCR). After washing twice with ice-cold PBS, cells were lysed using TRIzol reagent and the total RNA was extracted according to the manufacturer protocol (Invitrogen Life Technologies). cDNA synthesis were performed on RNA (1 μg) using Super Script III First-Strand Synthesis Super Mix kit (Invitrogen Life Technologies). qRT-PCR was carried out in a Roter Gene 3000 (Corbett Life Science, Mortlake, Australia) using SYBR Green ER qPCR Super Mix Universal kit (Invitrogen Life Technologies) as per supplier protocol.

Primer sequence of genes under study are given in table below [14].

Gene	Sequence	Product size (bp)
rps9 (housekeeping gene)	5′-CGTCTCGACCAAGAGCTGA-3′, 5′-GGTCCTTCTCATCAAGCGTC-3′	133
bcl-2	5′-AGGAAGTGAACATTTCGGTGAC-3′, 5′-GCTCAGTTCCAGGACCAGGC-3′	146
bax	5′-TGCTTCAGGGTTTCATCCAG-3′, 5′-GGCGGCAATCATCCTCTG-3′	170

Amplification reaction was as follows: pre-incubation at 50 °C for 2 min followed by denaturation at 95 °C for 10 min and 40 cycles at 95 °C for15 s, annealing and extension at 60 °C for 20 s and 72 °C for 15 s were performed with Roter Gene 6 (Corbett Life Science). Data obtained are expressed as CT which is the PCR cycle number at which the accumulated fluorescent signal in each reaction crosses a threshold above background. Relative Bcl-2 or Bax RNA amount was calculated as follows: ΔCT calibrator = CT [gene of interest (calibrator)] − CT [house keeping gene (calibrator)], ΔCT sample = CT [gene of interest (sample)] − CT [house keeping gene (sample)] ΔΔ = (ΔCT calibrator − ΔCT Sample). Relative RNA amount was compared to the control by using the formula $=2^{-\Delta\Delta CT}$.

2.7. Mcl-1 and p53 determinations

Western blotting was performed on equivalent amounts of extracts of cell lysates of CEM and CEM/dEpoB300. Western blotting for Mcl-1 was carried out according to the manufacturer instructions (R&D Systems, Minneapolis, USA). To prepare total cell lysates, cells are solubilized in hot 2× SDS gel sample buffer (20 mM dithiothreitol, 6% SDS 0.25 M Tris, pH 6.8, 10% glycerol, and bromophenyl blue) at 2×10^6 to 1×10^7 cells/ml. The extracts are heated in a boiling water bath for 5 min and then sonicated with a probe sonicator with 3–4 burst of 5–10 s each. Lysates from equal amounts of total cells were electrophoresed on a 12% polyacrylamide gel followed by transfer to a polyvinyl diene difluoride membrane. The membrane was blocked in 2% non-fat dry milk in TBST and incubated with the primary antibody, for 1 h with a monoclonal anti-Mcl-1 antibody (diluted 1.5 μg/ml in 1% non-fat dry milk in TBST) overnight. The secondary antibody incubation was 1 h for Mcl-1 detection, a peroxidase-conjugated goat anti-rabbit IgG (Santa Cruz, Australian subsidiary, Sydney) diluted 1:10,000 in TBST containing 2% non-fat dry milk was used. The bands corresponding to Mcl-1 were detected using a chemiluminescence reagent (Perkin Elmer Cetus, Foster City, CA, USA).

An ELISA kit was used to quantify p53 levels in samples (Human p53 Immunometric Assay Kits; Assay Designs, Sydney, Australia) according to the manufacturer instructions.

2.8. Statistical analysis

All calculations and statistical analysis were carried out using Graph Pad Software (Graph Pad Prism version 3.0, San Diego, CA, USA). Data are presented as the mean ± S.E.M. for the indicated number of experiments. Depending on the test performed, samples were run in replicates of 4 or 8 and each experiment was performed at least twice. Statistical analysis of data was performed using one-way analysis of variance (ANOVA), followed by the post hoc Tukey test. In all statistical evaluations $P < 0.05$ was used as the criterion for statistical significance.

3. Results

3.1. Differential effects of PTX and ABZ on cell proliferation rate

Treatment of CEM and CEM/dEpoB300 with PTX led to concentration-dependent inhibition of cell proliferation (Fig. 1A). IC_{50} (concentration inhibiting cell proliferation by 50%) values for PTX in CEM and CEM/dEpoB300 were calculated to be 2.86

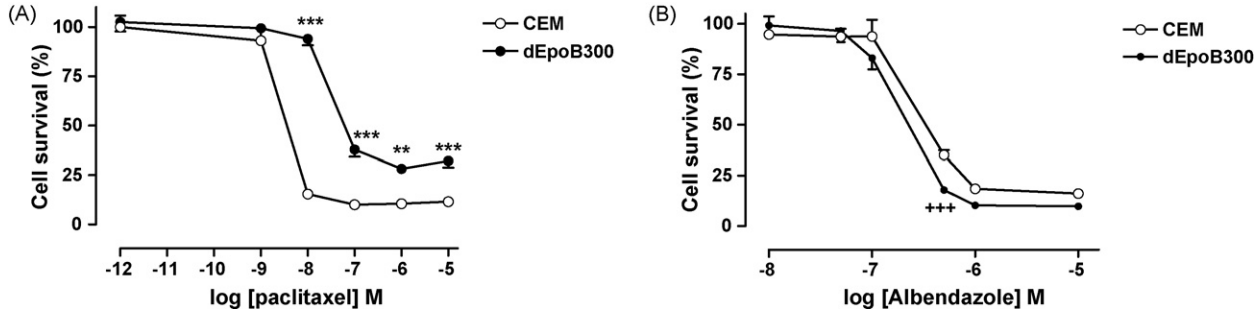

Fig. 1 – Inhibition of resistant cells by ABZ. Concentration-dependent inhibition of proliferation of CEM and CEM/dEpoB300 cells *in vitro* by PTX (A) or ABZ (B). Cells were treated in culture for 3 days with various concentrations of PTX (1–10,000 nM) or ABZ (0.01–10 μM). Cell proliferation was measured using the MTT assay. Values (mean ± S.E.M.) represent percentage control of at least two determinations. $^{**}P < 0.01$ and $^{***}P < 0.001$ (CEM/dEpoB300 vs. CEM).

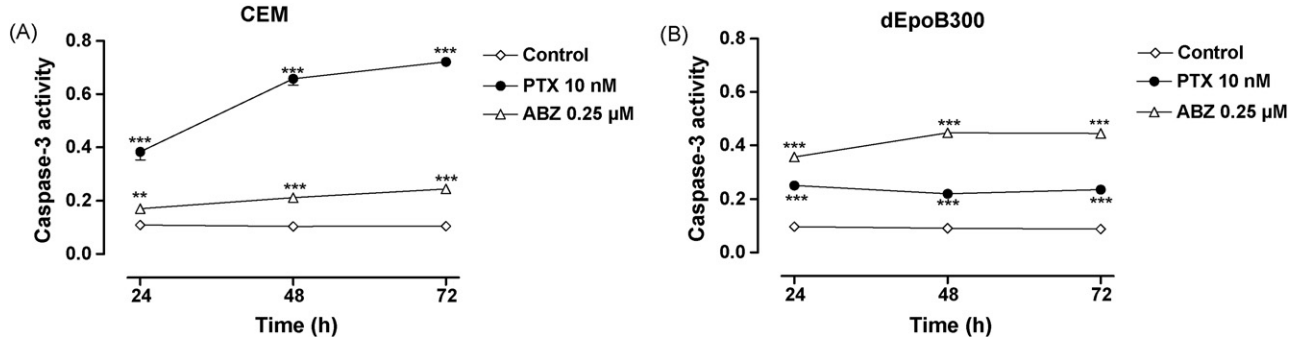

Fig. 2 – Induction of caspase-3 in resistant cells by ABZ. Caspase-3 activity in CEM and CEM/dEpoB300 cells treated with 10 nM PTX (A) or 0.25 μM ABZ (B) for 24, 48 and 72 h were measured using an ELISA. Values represent mean ± S.E.M. of two determinations; $^{***}P < 0.001$ compared to vehicle treated cells (control).

and 30.26 nM, respectively, confirming marked resistance of CEM/dEpoB300 cells to PTX ($P < 0.001$). In contrast to PTX, ABZ induced higher degree of inhibition in the CEM/dEpoB300 than in the parent CEM cells (Fig. 1B). The IC_{50} values obtained for ABZ in CEM and CEM/dEpoB300 cells were 0.32 and 0.16 μM, respectively. These results reveal for the first time that, in these cells, resistance to PTX does not confer resistance to ABZ and on the contrary the PTX-resistant cells were significantly more sensitive to the anti-proliferative effects of ABZ. This observation may be of high importance in malignant cells resistant to PTX or other tubulin polymerizing agents.

3.2. *Differential effects of PTX and ABZ on caspase-3 activity*

To investigate if the anti-proliferative effects observed with PTX and/or ABZ is a consequence of changes in the caspase-3 activation, caspase-3 assay was performed. In CEM cells, PTX (Fig. 2A) treatment led to time-dependent increased caspases-3 activity with a 12-fold increase recorded at the end of the incubation period (72 h). However, CEM/dEpoB300 cells responded to 72 h PTX treatment with only a 3-fold increase in caspase-3 activity, when compared to the vehicle treated cells (Fig. 2B). This is a significant reduction ($P < 0.001$) in PTX induced caspases-3 activity in these PTX resistant cells. On the other hand, compared to control, ABZ treatment led to enhanced production of caspases-3 activity, 2-fold in CEM cells and 7-fold in CEM/dEpo300 cells (Fig. 2A and B, respectively). Data obtained show that treatment with PTX results in a caspases-3 level ratio of 3/12 in CEM/dEpoB300 to CEM, while the ratio after ABZ treatment stands at 7/2. In line with the cell proliferation assay results, compared to the parent CEM cells, the PTX-resistant CEM/dEpoB300 cells exhibited profound hyper-sensitivity to the pro-apoptotic effects of ABZ.

3.3. *Cytochrome c release, in response to PTX and ABZ*

Release of mitochondrial cytochrome c has been shown to activate a chain of events leading to caspase-3 activation and apoptosis. We therefore investigated the effect(s) of PTX and ABZ on mitochondrial cytochrome c release. Treatment with either PTX or ABZ led to increased cytosolic cytochrome c levels in a time-dependent manner. At 72 h, compared to control, there was a 6-fold increase in cytochrome c release in PTX treated CEM cells and a 2-fold increase in CEM/dEpo300 cells (Fig. 3A and B). At the same time point, cytochrome c levels in ABZ treated cells were 0.75-fold in CEM and 1.6-fold in CEM/dEpo300 cells (Fig. 3A and B). Again, the results reveal that while PTX has potent effects on the parent cell CEM, its

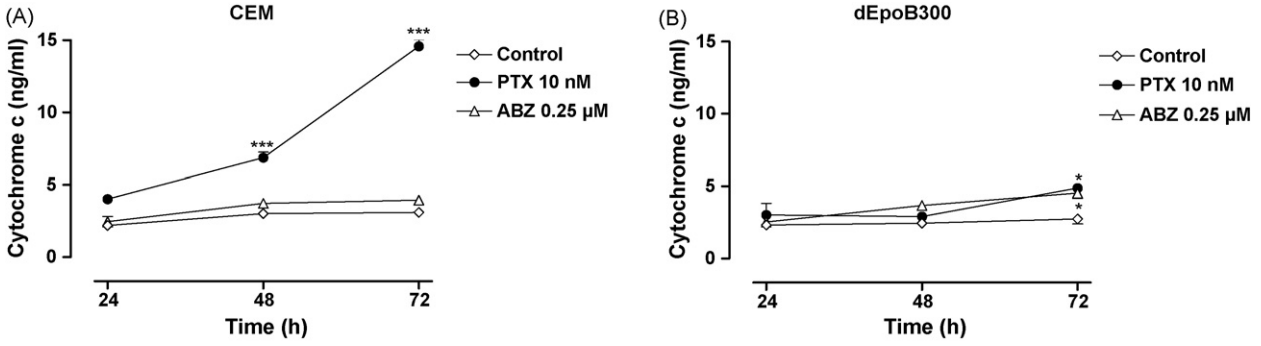

Fig. 3 – Release of cytochrome c by ABZ. Cytochrome c levels in CEM (A) and CEM/dEpoB300 (B) cells in culture following 24, 48 or 72 h treatment with PTX or ABZ. Values are (mean ± S.E.M.) of two to three determinations. $^{*}P < 0.05$, $^{***}P < 0.001$ compared to control values.

ability to stimulate cytochrome c release in the CEM/dEpoB300 cells has been dramatically reduced. On the contrary, ABZ has a greater effect on the resistant sub-line than on the parental cells. These results indicate that, as a consequence of epothilone treatment, CEM cells have acquired mutation(s) making them more sensitive to the pro-apoptotic effects of ABZ.

3.4. *Bcl-2 and Bax level, in response to PTX and ABZ*

Using ELISA, initially, we measured levels of Bcl-2 and Bax in parental and the resistant sub-line, without drug treatment. Bcl-2 levels were about 3.5-fold higher in CEM/dEpoB300 than in CEM cells, whereas, levels of the pro-apoptotic protein Bax were, 10 times higher in CEM than in its resistant sub-line CEM/dEpoB300. Treatment with either PTX or ABZ did not significantly change the Bcl-2 levels in CEM cells, whereas, in CEM/dEpoB300 cells, PTX and ABZ reduced Bcl-2 levels by 50.6 and 63.4%, respectively (Fig. 4A and B). In addition, both PTX and ABZ significantly enhanced Bax protein levels in these cells (Fig. 4C and D). However, what seems to be more important in terms of an apoptotic event is the ratio of the Bcl-2 to Bax rather than the levels of each protein individually [15]. Comparison of the Bcl-2 to Bax ratios revealed that, in PTX-sensitive CEM cells the ratio does not change while in the CEM/dEpoB300 resistant cells both PTX and ABZ treatment significantly change the ratios in favour of apoptosis. To determine whether ABZ or PTX affect Bcl-2 and/or Bax mRNA, using Real Time PCR, we examined Bcl-2 and Bax mRNA levels before and after PTX or ABZ treatment. Both drugs reduced Bcl-2 mRNA levels in CEM (Fig. 4E) and CEMdEpoB300 cells (Fig. 4F). Whereas in CEM, PTX was more effective in lowering mRNA levels, in dEpoB300 cells it was ABZ treatment which led to the more profound effect at 48 and 72 h. On the other

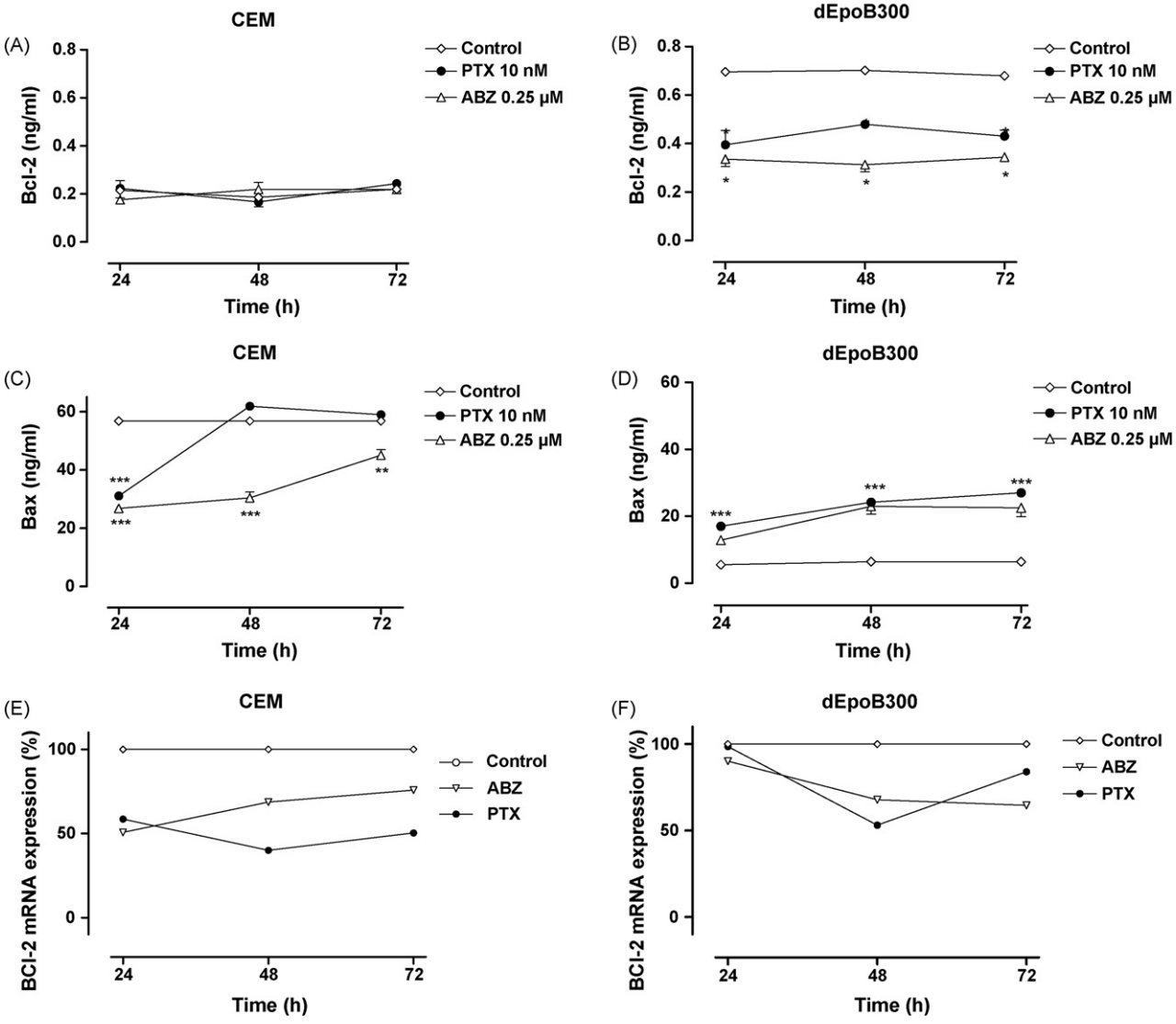

Fig. 4 – Effects of ABZ on Bcl-2 and Bax. Measurement of Bcl-2 (A and B) or Bax (C and D) protein and RNA levels in PTX or ABZ treated (24, 48 or 72 h) CEM and CEM/dEpoB300 cells. Proteins were measured by standard ELISA kits and real-time polymerase chain reaction was used to measure Bcl-2 mRNA levels. Cells were quantified and expressed relative to the vehicle treated controls (E and F).

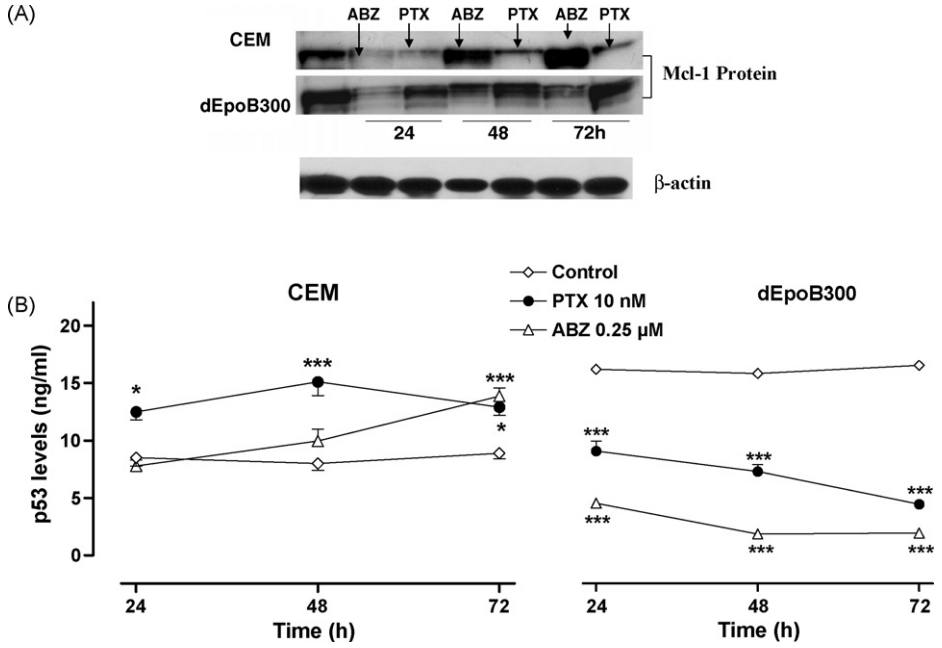

Fig. 5 – Reduction of Mcl-1 and mutant p53 levels by ABZ. Western blot analysis showing Mcl-1 protein in CEM and CEM/dEpoB300 cells (A). Cells treated with PTX (10 nM) or ABZ (0.25 μM) for 24, 48 or 72 h were also analysed for p53 levels using ELISA (B). Values represent mean ± S.E.M. of two determinations. $^{*}P < 0.05$, $^{***}P < 0.001$ compared with values for the vehicle treated cells.

hand, at the concentrations employed, neither PTX nor ABZ had any significant effect Bax mRNA levels.

3.5. *Elevated Mcl-1 and p53 levels in resistant cells*

We next examined the myeloid cell factor-1 (Mcl-1 protein) in these cells. Mcl-1 is a death-inhibiting member of the Bcl-2 family with elevated levels in many cancers including ALL [16]. Cells treated up to 72 h with either ABZ (0.25 μM) or PTX (10 nM) were analysed for Mcl-1 protein by Western blotting (Fig. 5A). Consistent with the Bcl-2 results, the Mcl-1 levels were found to be profoundly higher in the CEM/dEpoB300 cells, than in CEM cells. In PTX treated CEM cells, Mcl-1 protein was reduced in a time-dependent manner. In ABZ treated cells, down-regulation of Mcl-1 after 24 h was followed by increased levels of the protein at 48 and 72 h. Treatment with PTX led to slightly lowered levels at 24 and 48 h but a strong band appeared at 72 h indicating that, in resistant cells, PTX may not be effective in lowering Mcl-1 protein. Treatment of CEM/dEpoB300 with ABZ (0.25 μM) led to consistently lower Mcl-1 levels at all time points treated. The ABZ effect on lowering Mcl-1 was most evident in the 24 h treated cells.

The p53 data obtained in response to PTX and ABZ treatment are depicted in Fig. 5B. Compared to 8923 pg/ml in CEM cells, the p53 levels in CEM/dEpoB300 cells were found to be18,910 pg/ml ($P < 0.001$). In resistant cells, treatment with PTX or ABZ led to significant time-dependent reduction of p53 levels. ABZ was significantly more effective in lowering p53 (mutant) levels than PTX. These results follow the pattern seen with other mediators where, ABZ was found to be consistently more effective in the PTX-resistant CEM/dEpoB300 cells than in the parent PTX-sensitive CEM cells.

4. Discussion

It has been shown that the β-tubulin is the site where many anticancer agents bind to and thereby interfere with microtubulin dynamics leading to a broad spectrum of activity against both haematological malignancies and solid tumours [17]. Down stream events from tubulin binding are believed to be critical events for the generation of apoptosis in the malignant cells [18]. Despite the clinical success of taxanes as the most effective anticancer agents, the emergence of drug-resistant tumour cells hampers the chances of a complete curative effect or continued therapeutic benefit [10]. To address the issue, identification of the factors that contribute to the ability of the cell to develop drug resistance is essential. It is believed that, perturbation of the orchestrated cascades of multiple interacting signalling pathways leading to cell survival and apoptosis may contribute (at least partially) to the emergence of drug resistance [19].

To understand the mechanisms behind resistance of CEM/dEpoB300 to PTX and to find out its response to ABZ (a depolymerizing agent), the present study was undertaken. In culture, both PTX and ABZ reduced the cell proliferation of CEM and CEM/dEpoB300 cells in a concentration-dependent manner. However, compared to CEM, to inhibit CEM/dEpoB300 cells, approximately 10-fold higher concentrations of PTX were required. In contrast, it was found that in comparison to CEM, the CEM/dEpoB300 cells were significantly more sensitive to the anti-proliferative effects of ABZ.

The activation of caspases plays a pivotal role in the execution of cell apoptosis. Recent studies have demonstrated that caspase-3 is the major caspase activated in response to distinct apoptotic stimuli [20–23]. Our results show that

both PTX and ABZ increase caspase-3 activity in CEM and CEM/dEpoB300 cells albeit to different extent. In case of PTX, there was a profound decline in response from CEM to CEM/dEpoB300. In contrast, resistant cells treated with ABZ had higher caspases-3 levels. The enhanced response of the resistant cells to ABZ is in line with the proliferation data.

It has been shown that the release of cytochrome *c* from mitochondria into the cytosol triggers the induction of caspases leading to apoptotic cell death [24,25]. Compared to CEM/dEpoB300, our results demonstrate a 3-fold increase in cytosolic cytochrome *c* in CEM cells in response to PTX treatment. In contrast, cytochrome *c* levels produced in response to ABZ treatment by CEM/dEpoB300 cells were significantly higher ($P < 0.001$) than those found in the cytosol of the CEM cells.

Mitochondrial release of cytochrome c is highly regulated by the Bcl-2 family of proteins, which include the death agonists Bax, Bak, Bad and Bcl-Xs, and antagonists Bcl-2, Bcl-w, Bcl-xL and Mcl-1 [26–28]. It has been reported that PTX turns the balance of pro-apoptotic and anti-apoptotic proteins toward apoptosis by upregulation of Bax expression [29], translocation of Bax from cytosol to mitochondria [30] and hyperphosphorylation of the Bcl-2 protein [31]. Here in our investigation, in CEM cells, PTX or ABZ treatment did not reduce Bcl-2 protein concentrations, while in the CEM/dEpoB300 cells, both PTX and ABZ reduced Bcl-2 and increased the Bax concentrations leading to changed Bcl-2/Bax ratios. Variation in Bcl-2 to Bax ratio in cell usually reflects a variation of the apoptotic status. An anti-apoptotic state is associated with a high ratio whereas a pro-apoptotic state is favoured when this ratio is decreased [15,32]. Heterodimerization of Bcl-2 with Bax appears to be critical in preventing Bax mediated apoptosis [32,33]. Overexpression of Bcl-2 and increased Bcl-2/Bax ratio in chemo-resistant cancer cells has previously been well documented in a number of common human malignancies, including lung, ovarian, breast and prostate cancers [34–36].

Mcl-1 is another anti-apoptotic Bcl-2 family protein which has increased expression in hematological malignancies [37]. Here, we found that Mcl-1 level was higher in the PTX-resistant than in the PTX-sensitive cells. In addition, incubation of CEM cells with ABZ led to marked reduction of Mcl-1 protein levels at 24 h and highly increased levels at 72 h. It is likely that elevated Mcl-1 expression is associated with increased protection from cell death [38]. Compared to CEM, in the CEM/dEpoB300 cells the PTX effect on Mcl-1 protein was highly diminished while that of the ABZ was extended up to 72 h. It is quite evident that ABZ is profoundly more effective in reducing antiapoptotic proteins in the PTX-resistant than in PTX-sensitive cells.

The strong genetic and biochemical tie between the p53 and the Bcl-2 family of proteins is of fundamental importance to cancer biology [39]. A negative regulation of Bcl-2 transcription by the p53 tumour suppressor gene has been reported [40]. CEM cells express mutant p53 [41] and here we found that the p53 protein levels were twice as high in the CEM/dEpoB300 cells than in the CEM cells. This is consistent with reports showing that p53 level is frequently higher in resistant cells [18,42].

In summary, these results demonstrate for the first time that ABZ is a highly effective agent in suppressing proliferation of the leukemic cells CEM and even more profoundly in suppressing its paclitaxel resistant sub-line CEM/dEpoB300 cells. In these cells, the anti-proliferative effects correlated well with the ABZ induced pro-apoptotic changes.

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