Protection from Adriamycin Cytotoxicity in L1210 Cells by Brefeldin A

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ABSTRACT

We present studies which suggest that the cytotoxic action of Adriamycin (ADR) may involve intracellular pathways of vesicular transport. The movement of proteins or lipids from the endoplasmic reticulum to the plasma membrane via the Golgi organelle and associated compartments exhibits several temperature-sensitive steps between 15°C and 20°C. In this same temperature range, ADR loses its cytotoxic capacity. Using the inhibitor brefeldin A (BFA), we have investigated the possible role of intravesicular trafficking in the loss of ADR activity and the induction of protection from cytotoxicity at low temperature in L1210 cells. We show here that cells pretreated at 37°C for 2 h with BFA could be protected from a subsequent exposure to ADR. The concentration causing 50% inhibition, determined by cloning in soft agar, was increased approximately 3.5 fold. L1210 cells could also be protected from the topoisomerase II inhibitors etoposide and amssacrine, but to a lesser extent; the concentration causing 50% inhibition for the latter inhibitors were increased 2-fold. Spectrofluorometric analysis of intracellular ADR accumulation revealed that there was no significant difference in the level of ADR in cells with or without BFA pretreatment. In addition, examination of ADR-induced cleavable complex formation by alkaline elution showed no significant difference in the level of DNA strand breaks in cells which had been pretreated with BFA even though there was a large difference in survival. Further examination of the persistence of DNA damage after a period of up to 6 h of repair revealed that cells which were pretreated with BFA removed DNA strand breaks at rates equivalent to those of cells which had received ADR directly. These results suggest that the protective effect induced by brefeldin A does not involve uptake, DNA damage, or repair but instead implicates protein or lipid interactions which may be independent of DNA damage and which may influence critical events that take place after the topoisomerase II-DNA complex has been formed.

INTRODUCTION

Previous work in our laboratory has investigated the reversible property of the temperature-dependent cytotoxic action of ADR.3 Below 20°C, ADR loses its ability to kill cells even though potentially toxic levels of the drug can still be accumulated within the cell (1). Once cells are raised above 20°C, however, the cytotoxic process ensues. Concomitant with the loss of activity is the inability to detect DNA damage in cells treated with ADR at temperatures below 20°C (2), suggesting a requirement for DNA damage leading to drug-induced cytotoxicity. These studies also revealed that the protective process is unique to whole cells and may involve proteins of extranuclear origin since isolated nuclei accumulated ADR-induced DNA strand breaks in a dose-dependent fashion at low temperature. Furthermore, we have shown that the cytotoxic process can be inhibited in whole cells by sequestration of extracellular drug with high molecular weight DNA (1). The induction of complete cellular protection in the presence of cytotoxic concentrations of intracellular drug supports the role of cell surface interactions as an important component in the initiation of cell death by ADR.

Proteins present in the plasma membrane often require recycling and/or modification to maintain an active function. If there are indeed membrane proteins the interactions of which with distal sites are important for the cytotoxic process initiated by ADR, then these interactions should be susceptible to disruption by inhibiting intracellular protein modification or accessibility to specific sites. It has already been demonstrated that the cytotoxicity of several topoisomerase II inhibitors can be diminished by pretreatment of cells with inhibitors of protein synthesis (3–5). However, no individual protein, other than topoisomerase II, has been unambiguously identified as being decisive in mediating the action of ADR. We summarize in this report experimental studies describing the influence of disruption of normal protein and lipid transport on the actions of ADR.

MATERIALS AND METHODS

Cells. Murine leukemia L1210 cells were grown in suspension in McCoy’s 5A medium (GIBCO, Grand Island, NY) containing l-glutamine at 2 mM and 10% heat-inactivated horse serum (GIBCO). The cells were maintained in a humidified atmosphere at 37°C and 10% CO2 and passed twice each week. The doubling time for L1210 cells was approximately 15 h. All experiments were performed on log phase cells (200,000–500,000 cells/ml).

Chemicals. ADR at 20 mg/ml and m-AMSA at 9.8 µg/ml were purchased from Sigma, dissolved in 70% ethanol, and maintained at ~20°C. Dilutions used in cell treatments were made into 70% ethanol or McCoy’s 5A medium. Brefeldin A (Sigma) at 20 mg/ml was dissolved in 100% methanol and stored at ~20°C. Taxol, supplied by the National Cancer Institute, was dissolved at 20 mg/ml in dimethyl sulfoxide, as was nocodazole (Sigma) at 1 mg/ml, and maintained at ~20°C. Etoposide at 59 µg/ml was dissolved in 70% ethanol and maintained at ~20°C.

Cloning Assay for Cell Survival. Survival of cells after a given treatment was assessed by cloning in soft agar. The method has been published previously (1). Briefly, following a given treatment cells were washed three times in complete medium and resuspended at a concentration of 100,000 cells/ml. These cell suspensions were then serially diluted 1:20 (10 ml), followed by 1:10 (3 ml) into complete McCoy’s 5A medium. The entire 1:10 dilution was added to 27 ml of “cloning medium” containing McCoy’s 5A medium with a final concentration of 15% horse serum, 2 mM l-glutamine and 0.42% Difco Bacto-Agar. The cell suspension was aliquoted into five 60-mm tissue culture dishes/condition (Corning), 6 ml/dish, and allowed to gel at room temperature. The 1:20 dilution for each condition was counted directly using a Coulter Counter to determine the number of cells plated/condition. Culture dishes were grown for 10–12 days in a humidified incubator at 37°C and 10% CO2. Colonies were counted, and the average cloning efficiencies were determined for each condition. Survival was calculated by comparison to untreated controls.

Uptake of ADR. ADR was quantitated by fluorescence spectroscopy using an SLM 4800 instrument. Approximately 2.5–3.5 × 108 log phase L1210 cells were concentrated to 2–3 × 106 cells/ml and treated with or without BFA (10 μM) for 2 h at 37°C. Initial treatments were then divided into equal aliquots (10 ml) and the cells were treated ± ADR for 2 h at 37°C. Cells were centrifuged at 500 × g for 5 min at 4°C, and the cell pellets were washed 3 times in ice-cold PBS (pH 7.4). The cell pellet was then resuspended in 2 ml of Na2CO3 (pH 9.0), and the lystate was transferred to glass 18 mm × 150 mm test tubes. ADR was extracted from the lystate by three successive additions (3 ml each) of an organic buffer containing ethyl acetate and n-propyl alcohol in a ratio of 9:1, respectively. Tubes were vortexed vigorously and then centrifuged at 1000 × g for 15 min at 4°C to efficiently separate the organic layer from the water layer. The organic layer was removed and pooled for each condition. These were then dried under N2 and the residues were resuspended in 2 ml of ethanol at 70%. ADR content for each condition was determined by fluoro-
ence and comparison to known standards. Excitation was at 470 nm and the emission was collected using a 550 nm long-pass filter.

Alkaline Elution. Total DNA strand breaks were assayed using alkaline elution as described elsewhere (6). Forty ml of L1210 cells at 5 x 10^6 cells/ml in complete McCoy's 5A medium were labeled with either 0.1 μCi/ml of [3H]thymidine (ICN) or 0.1 μCi/ml of [4C]thymidine (ICN) for 24 h at 37°C and 10% CO2. The cells were then centrifuged and resuspended in 50 ml of complete McCoy's 5A medium without radiolabeled nucleotide and chased for approximately 18 h. The cells labeled with [3H]thymidine were then counted using a hemacytometer and treatments were performed. Following all treatments, the cells were washed with ice-cold PBS-6 mM EDTA (pH 7.4) and resuspended at 1 x 10^6 cells/ml in cold PBS-6 mM EDTA. An aliquot of untreated [3H]labeled cells and a separate aliquot of PBS-washed, [3H]labeled cells were exposed to 400 rads of ionizing radiation using a 60Co or 137Ce source. The irradiated cells labeled with [3H] served as a calibration curve for each experiment, while the irradiated [3H]labeled cells served as an internal standard for each condition. 5.0 x 10^5 [3H]labeled cells, with or without treatment, and 5.0 x 10^3 [4C]labeled cells exposed to 400 rads were added onto 2.0 μm polycarbonate filters. The cells were washed on the filters with 5 ml of cold PBS-6 mM EDTA and then lysed for 1 h at room temperature with lysis buffer containing 2% sodium dodecyl sulfate, 30 mM disodium EDTA, and 0.5 mg/ml proteinase K (pH 10.1). Lysates were washed with 5 ml of 20 mM disodium EDTA (pH 10.1). The DNA was then eluted overnight with 20 ml of elution buffer containing 0.1% sodium dodecyl sulfate, 2.0% tetrapropylammonium hydroxide, and 20 mM EDTA (free acid; pH 12.2). Eight 2.5-ml fractions were collected/condition. Twelve ml of Ecolume (ICN) were added to each fraction and the [3H] and [4C] content was quantitated on a Packard 2500TR liquid scintillation counter. The polycarbonate filters from each condition were dissolved in 750 μl of Protosol (NEN) and the isotope content was quantitated by addition of 5 ml of Ecolume and liquid scintillation counting. The data were graphed as the percentage of [3H]thymidine retained on the filter versus the percentage of [4C]thymidine retained on the filter. Calculations for single-strand breaks (SSB) were quantitated using [4C] values obtained for each condition when 40% of the internal standard ([3H] + 400 rads) remained on the filter.

SSB = \frac{\log(\text{treated}/\text{untreated})}{\log(\text{X-ray}/\text{untreated})}

The results were expressed in rad equivalents by multiplying the frequency of single-strand breaks by the dose of radiation, in rads, of the calibration curve. For all experiments, this was 400 rads.

Repair of DNA Damage. The extent of DNA breaks remaining after 3 or 6 h of repair was assessed using the alkaline elution technique described above with the following modifications. After treatment of [4C]labeled cells with or without 10 μM BFA and 0.14 μM ADR, the cells were washed 3 times in chilled PBS with or without 10 μM BFA. The cells were then resuspended in complete McCoy's 5A medium with 10% horse serum and allowed to repair at 37°C for up to 6 h. The cells were then centrifuged and resuspended at 1 x 10^6 cells/ml in PBS-6 mM EDTA and layered on 2.0 μm polycarbonate filters (Nucleopore). The cells were washed and lysed, and the DNA was eluted as described in "Alkaline Elution." The calibration line for repair experiments was created using [4C]- and [3H]-labeled cells which had been exposed to 450 rads of ionizing radiation from a 137Ce source.

Measurement of DNA Synthesis. Log phase L1210 cells were centrifuged and resuspended at 1 x 10^6 cells/ml in complete McCoy's 5A medium with L-glutamine and 10% horse serum. BFA (10 μM) was added and the cells were aliquoted into 96-well plates at 100 μl/well. The cells were incubated for 2 h at 37°C before addition of [3H]thymidine at 1 μCi/well. The cells were incubated for an additional 2 h at 37°C, and the level of incorporation of [3H]thymidine was determined at various intervals. Cells were harvested onto glass fiber filters and the content of [3H]thymidine was determined using a Packard Matrix 96.

RESULTS

Effect of BFA on ADR-induced Cytotoxicity. If protein transport processes contribute to ADR-induced cytotoxicity, then the degree of cytotoxicity induced by a given concentration of ADR should be reduced by disruption of vesicular movement. To test this hypothesis, L1210 cells were pretreated for 2 h at 37°C with 10 μM BFA. The fungal metabolite BFA has been reported to reversibly disrupt anterograde vesicular trafficking between the ER and the Golgi apparatus (7) by inducing the dissociation of a M1, 110,000 protein critical for vesicular budding and fusion from the membrane of the Golgi organelle (8). After pretreatment with BFA, the cells were subsequently exposed to various concentrations of ADR for 2 more h at 37°C in the presence of BFA and cell survival was determined by cloning in soft agar. As shown in Fig. 1A, the percentage survival was significantly increased if cells were pretreated with BFA prior to an exposure to ADR. The concentration causing 50% inhibition was increased from ~0.03 μM to 0.1 μM.

The protection that cells acquired from ADR cytotoxicity was partially dependent on the concentration of BFA at which cells were pretreated. Fig. 1B indicates that the cell survival increased in cells pretreated for 1 h with increasing concentrations of BFA prior to a 2-h exposure to 0.1 μM ADR. Maximal protection was attained by pretreatment with 100 μM BFA, although the effect was not dramatically increased over lower concentrations (10 μM). BFA by itself was not toxic at the concentrations and times used. It is important to note, however, that although low temperature can provide complete protec-

![Figure 1](https://cancerres.aacrjournals.org)

**Fig. 1.** (A) Effect of brefeldin A pretreatment on Adriamycin-induced cytotoxicity. L1210 cells at 2-4 x 10^6 cells/ml were pretreated with 10 μM BFA for 2 h at 37°C. The cells were then exposed to various concentrations of Adriamycin for an additional 2 h at 37°C and washed, and the cell survival was measured by cloning in soft agar. Data represent the sum of 5 independent experiments with 5 replicates/data point for each experiment. (B) Influence of increasing concentrations of brefeldin A during pretreatment on the protection from ADR cytotoxicity. L1210 cells were pretreated with various concentrations of BFA for 1 h at 37°C. The cells were then exposed to 0.1 μM ADR for 2 h at 37°C and washed; the percentage survival was determined by cloning in soft agar. The average survival of nonpretreated cells was 8.3%. Data represent the mean of 3 independent experiments each with 5 replicates/data point. Bars, SD.
tion from any concentration of ADR, the protection provided by BFA was not absolute and could be completely overwhelmed at ADR concentrations >0.25 μM.

We also examined the effect of BFA on drug-induced cytotoxicity of the additional topoisomerase II inhibitors etoposide and m-AMSA. Fig. 2 indicates that cells could also be protected from etoposide and m-AMSA by preincubation with 10 μM BFA for 2 h. The concentration causing 50% inhibition for both agents was increased approximately 2-fold. This protection was not as exaggerated as that observed for ADR, however, and may be a reflection of the manner with which each of these agents interacts with the cell.

Influence of Nocodazole and Taxol on ADR Cytotoxicity. In order to test if microtubule-based transport is involved in the induction of ADR cytotoxicity, L1210 cells were pretreated with 5 μM nocodazole, an inhibitor of microtubule polymerization (9), for 1 h at 37°C before exposure to ADR for an additional 2 h at 37°C. Nocodazole was also present during exposure to ADR. Survival was assayed by cloning in soft agar. The results illustrated in Fig. 3A show that pretreated cells had an increased survival of approximately 2-fold when subsequently exposed to ADR. Although the disruption of microtubule formation can result in the inhibition of other cellular processes in addition to protein transport, most notably mitosis, there is no evidence to suggest that such inhibition would lead to protection from ADR-induced cell death. ADR has, by itself, been shown to cause a blockade of cells in the G2-M phase of the cell cycle (10–13).

Stabilization of microtubules has been demonstrated to be an important component of the activity of the antitumor agent taxol (14–16). At low temperature, cytoplasmic microtubules have been shown to be unstable, losing their filamentous organization (17, 18). This can be prevented in whole cells by pretreatment with taxol (14, 16, 19). These stabilized microtubules are resistant to depolymerization induced by low temperature as well as drugs. We hypothesized that if destabilization of microtubules contributed toward the protection from ADR which cells acquired below 20°C, then perhaps by maintaining microtubules at low temperature we might overcome the protective process. We therefore attempted to stabilize microtubules by addition of taxol prior to an exposure to ADR. Fig. 3B illustrates that pretreatment with 40 μM taxol did not significantly alter the protection cells acquired from ADR at 0°C. L1210 cells were pretreated with taxol for 30 min at 37°C. The cells were transferred to 0°C for 2 h, exposed to ADR for an additional 2 h at 0°C, washed, and cloned in soft agar. Data represent the mean of 3 independent experiments with 5 replicates/data point. Bars, SD.
with DNA. Using alkaline elution, we examined the amount of total ADR-induced single-stranded DNA breaks in cells which had been pretreated with 10 μM BFA for 1 h, prior to a 2-h exposure to various concentrations of ADR. At all ADR concentrations examined, there were no significant differences in the formation of strand breaks in BFA-pretreated cells compared to cells receiving ADR directly (Fig. 5).

Persistence of ADR-induced DNA Damage. The ability of BFA-pretreated cells to accumulate levels of DNA damage similar to those of nonpretreated cells suggests that involvement of interactions which may occur independent of DNA damage, or subsequent to the formation of the cleavable complex, may have a critical role in whether the cell dies. The persistence of DNA damage is one aspect which we thought warranted investigation. It is possible, for example, that DNA damage accumulated under BFA may be more readily removed or repaired, thereby not contributing as significantly toward the cytotoxic process. We therefore measured the levels of DNA strand breaks which remained after a period of repair. The results presented in Fig. 6 show that after 3 or 6 h of repair, equivalent percentages of initial DNA breaks remained in cells which had been pretreated with BFA compared to cells treated directly with ADR.

Role of DNA Synthesis in Protection by BFA and Cytotoxicity Induced by ADR. An additional area deserving investigation was the effect that BFA may have on the ability of the cell to maintain DNA synthesis. Other laboratories have shown that the cytotoxicity of the topoisomerase agents m-AMSA and etoposide could be reduced if cells were pretreated with agents which inhibited nucleotide synthesis (20, 21). In order to test the possibility that BFA may be inducing protection via an alteration of the rate of DNA synthesis, L1210 cells were pretreated for 2 h at 37°C with 10 μM BFA and then allowed to incorporate [3H]thymidine for an additional 2 h at 37°C in the presence of BFA. The results illustrated in Fig. 7 show that BFA did not reduce the ability of L1210 cells to incorporate labeled nucleotide.

**DISCUSSION**

ADR is a topoisomerase II inhibitor which has enjoyed considerable success in the clinic despite the lack of a full understanding of its mechanism of action. While the drug has been shown to cause DNA damage via stabilization of the topoisomerase II cleavage complex (22), there is incomplete information describing various interactions which may regulate the ability of ADR to stabilize the topoisomerase

![Graph showing the influence of BFA pretreatment on the level of topoisomerase-mediated DNA damage induced by ADR.](image)

**Graph:** Influence of BFA pretreatment on the level of topoisomerase-mediated DNA damage induced by ADR. L1210 cells, which had been labeled with [3H]thymidine, were exposed to 10 μM BFA for 1 h at 37°C. The cells were then exposed to various concentrations of ADR for 2 h at 37°C and washed; the level of total DNA strand breaks were measured using alkaline elution. Data represent the mean of 3 independent experiments. Bars, SE.
II-DNA complex as well as information illustrating events which take place after the DNA has been compromised. When considering
the many different actions of ADR in addition to the ability to damage
dNA (23–25), it has been difficult to assert a clear relationship be-
 tween the level of drug-induced topoisomerase-mediated lesions and cell death. It has been suggested that strand separation in the presence of
a stabilized, covalently bound topoisomerase II-drug complex
 could lead to erroneous reassociation of topoisomerase II subunits
and nucleotide tails generating nonhomologous recombination events
 and subsequent alterations in the DNA sequence (20). In accordance, it has
been demonstrated that the cytotoxicity of m-AMS and etoposide
 could be diminished in cells by pretreatment with inhibitors of nucleo-
tide synthesis such as aphidicolin and cordycepin (20, 21). However,
the protective effect initiated by inhibitors of nucleotide synthesis has
not been demonstrated for ADR. Furthermore, the protection that cells
acquired by pretreatment with BFA was not the result of inhibition of
nucleotide incorporation into DNA. Treatment of L1210 cells with 10
μM BFA did not inhibit DNA synthesis. Other laboratories have shown
that inhibition of protein synthesis (3–5) or depletion of intracellular
calcium (26) prior to exposure to topoisomerase II inhibitors results
in similar events as we have described here. In each case, the reduction
in protein synthesis or calcium levels was not accompanied by a
diminution in the formation of drug-induced cleavable complexes.

Intravesicular trafficking of proteins to the plasma membrane, or
from the plasma membrane to the interior, involves several steps,
many of which display a tightly regulated temperature dependence
(27). At temperatures below 20°C, internalization and degradation
rates of membrane proteins display a marked decline in activity (27–
29). The exit of proteins from the TGN to the cell surface is also
significantly reduced below 20°C (30, 31). At still lower temperatures,
such as 15°C, vesicles have been shown to accumulate in an inter-
mediate compartment between the ER and Golgi (27, 31). Stereologi-
 cal volume measurements of the Golgi compartment also indicate an
increase in the size of the TGN at 20°C and a decrease in the surface
area and volume of preceding Golgi compartments (32). Although
ADR has no identified receptor and diffuses freely into the cell at both
high and low temperature, albeit with reduced kinetics at low tem-
perature, there may be important regulatory proteins or cofactors
which are important in mediating cytotoxicity, yet with modification
or transport that is prevented by low temperature.

We used BFA and nocodazole in an attempt to disrupt cellular
transport mechanisms and measure the influence of such disturbances
on the ability of ADR to induce cell death. The drug BFA has been
shown to be extremely useful as a potent, reversible agent for disrup-
tion of vesicular transport processes (7, 33, 34). BFA induces a rapid
redistribution of the coat protein β-COP from the membranes of the
Golgi (8) resulting in the fusion of Golgi compartments, mixing of the
Golgi into the ER, and the subsequent inhibition of anterograde move-
ment of proteins from the ER (33).

Our rationale for using nocodazole derives from past research
which has supported a role for microtubules as an important com-
ponent in several membrane trafficking steps within the cell (35, 36).
In addition to helping maintain the organization of the Golgi apparatus,
 microtubules have been shown to be involved in several stages of
 protein movement, such as the transport of large endocytic vesicles
toward the nuclear region (37) as well as the movement of vesicles
between the ER and an intermediate or salvage compartment prior to
the Golgi (38, 39). The organization and kinetics of microtubules have
been shown to be dependent upon temperature. Observation of as-
sembly kinetics for in vitro tubulin preparations revealed a switch
from biphasic to monophasic if the temperature was decreased below
30°C (18). Immunocytochemical examination of frog oocytes cooled
to 25°C, 18°C, or 4°C indicated a progressive disruption or fragmenta-
tion of the spindle and displacement of chromosomes (40). A 2-h
low-temperature treatment at 0°C to 4°C was sufficient to disrupt all
but kinetochore microtubules in mitotic ptk1 cells (41). We therefore
reasoned that disruption of microtubule assembly and/or Golgi mor-
phology by low temperature may result in the partial loss of critical
architecture, microtubule-based vesicular transport, and Golgi func-
tion in L1210 cells; these losses may contribute toward the protection
from ADR acquired at low temperature. The fact that BFA provided
a consistently higher degree of protection from ADR than nocodazole
may be related to the degree to which each of these agents disrupts
various transport processes. For example, microtubules do not appear
to be involved in transporting newly synthesized proteins between
the salvage compartment, Golgi cisternae or trans-Golgi (35, 37). Fut-
thermore, Golgi units which had been disrupted with nocodazole were
shown to retain cis-trans polarity and functionality (42). Therefore,
although the addition of nocodazole to cells causes the depolymer-
ization of microtubules, the resulting influence on transport may be
limited to specific interactions such as fusion between early and late
endosomes (37), disassembly of the Golgi stacks (36), formation of

![Graph](image-url)

**Fig. 6.** Reversibility of ADR-induced DNA damage in cells with or without brefeldin A pretreatment. Log phase cells labeled with [14C]thymidine were pretreated for 1 h at 37°C with 10 μM BFA before exposure to 0.14 μM ADR for 2 h at 37°C. Cells were washed 3 times in PBS with or without 10 μM BFA and resuspended at 1 × 10^5 cells/ml in complete McCoy’s 5A medium at 37°C. The cells were allowed to repair for either 3 or 6 h and the levels of strand breaks remaining under each condition were assayed by alkaline elution. Results represent the mean of 4 independent experiments. Bars, SE.

![Graph](image-url)

**Fig. 7.** Effect of BFA on DNA synthesis. Log phase cells were concentrated to 1 × 10^6 cells/ml and exposed to BFA (10 μM) at 37°C for 2 h in 96-well plates (100 μl/well). [3H]Thymidine was added at 1 μCi/well and the cells were incubated for 2 h at 37°C. Incorporation of [3H]thymidine was assayed at 30- or 60-min intervals. The results represent the mean of an individual experiment with 12 replicates/data point. Bars, SD. These results were repeated in 3 additional experiments.
the TGN-early endosome (43), movement of newly synthesized proteins from the ER to the Golgi or from the Golgi to the plasma membrane, and recycling of receptors (34). As a result, microtubule-disrupting agents such as nocodazole may not universally inhibit the flow of membrane traffic but have instead been shown to result in the misdirection of vesicle movement (35). This lack of direction could prevent a signaling protein from reaching its intended destination or from being recycled to specific sites such as the plasma membrane or ER. Such alterations in vesicle movement may be sufficient to account for the limited protection from ADR observed in cells pretreated with nocodazole.

Protection from cell death by pretreatment with BFA is most likely related to the ability of BFA to inhibit some transport process rather than an unidentified property of the drug, although this latter explanation cannot be ruled out. The fact that temperature disrupts some of the same processes as BFA may also be coincidental; however, both conditions lead to protection from ADR-induced cytotoxicity. Temperatures around 20°C begin to selectively inhibit various steps within the transport pathways. Additional processes become inhibited as the temperature is lowered to 15°C. Decreasing the temperature below 20°C results in complete protection from ADR cytotoxicity. The protection provided by BFA at 37°C, although significant, is incomplete and may be related to the degree to which BFA can prevent the movement or modification of some critical protein. Low temperature presents an absolute block to protein movement while BFA causes a partial inhibition; e.g., BFA blocks anterograde transport from the ER to the Golgi but not retrograde transport from the plasma membrane to the TGN or from the Golgi to the ER.

The decreased level of protection provided by BFA against etoposide and m-AMSA compared to ADR may be a reflection of the cellular system (L1210) which we have used to conduct our studies or an indication of the manner in which each of these agents interacts with other cellular components in addition to DNA. The focus of this investigation was primarily on the interactions between BFA and ADR; however, another laboratory has shown that additional cell lines with other cellular components in addition to DNA. The focus of this investigation was primarily on the interactions between BFA and ADR; however, another laboratory has shown that additional cell lines with other cellular components in addition to DNA.

REFERENCES

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