Paclitaxel Induces Programmed Cell Death in MDA-MB-468 Human Breast Cancer Cells

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ABSTRACT

The ability of paclitaxel, one of the most active chemotherapeutic agents against breast cancer, to induce programmed cell death in hormone-independent MDA-MB-468 human breast cancer cells was assessed. Treatment of MDA-MB-468 cells led to growth inhibition, high-molecular-weight and oligonucleosomal DNA fragmentation, and apoptosis-associated morphological changes after either 3- or 24-h exposure to paclitaxel concentrations ≥10 nM. Additionally, cleavage products of poly(ADP-ribose) polymerase and lamin B1, two proteins that are cleaved early in the execution phase of programmed cell death, were detected. Quantitative studies indicated that exposure to paclitaxel for 24 h resulted in more DNA fragmentation than did 3-h exposure. Rapid induction of the early-response gene c-jun but not c-myc was associated with paclitaxel treatment. The ability of paclitaxel to induce high-molecular-weight DNA fragmentation and apoptosis-associated morphological changes in three other breast cancer cell lines was also established. These data suggest that paclitaxel, an agent known to stabilize microtubules and prevent cell division but not to act directly on DNA, induces programmed cell death in breast cancer cells.

INTRODUCTION

Breast cancer is the most common cancer in American women. New approaches to systemic therapy are needed, as age-adjusted mortality has not changed greatly for 50 years (1). Exploitation of pathways of programmed cell death (2-4), a mechanism of cellular suicide, is one potential approach. We have previously used human breast cancer cells to show that hormone-dependent MCF-7 cells growing in vivo undergo programmed cell death upon estrogen withdrawal (5). Furthermore, even hormone-independent breast cancer cells retain the ability to undergo programmed cell death in response to diverse agents, including fluoropyrimidines (6).

The critical event(s) necessary for activation of programmed cell death pathways have not yet been characterized, and specific targeting of a chemotherapeutic agent to these pathways is not yet possible. Increased understanding of the mechanisms by which existing chemotherapeutic agents act may lead to the development of new drugs or more effective use of the currently available ones. Paclitaxel (Taxol; Bristol-Myers/Squibb, Wallingford, CT) is one of the most active new agents currently used for the clinical treatment of breast cancer (7-10). It is known for its unique ability to stabilize microtubules, thus preventing chromosome segregation and subsequent cellular division. The goal of these studies was to determine whether paclitaxel, an agent that does not cause direct DNA damage, induces programmed cell death in human breast cancer cells.

MATERIALS AND METHODS

Cell Line and Culture Conditions. MDA-MB-468 cells were purchased from American Type Culture Collection (Rockville, MD) and maintained in improved minimal essential medium supplemented with 5% fetal bovine serum (Biofluids, Rockville, MD) and 2 mM glutamine. MCF-7, MDA-MB-231, and HS578T cells (obtained from Dr. Marc Lippman, Vincent T. Lombardi Cancer Center, Washington, DC) were maintained in DMEM supplemented with 5% fetal bovine serum and 2 mM glutamine. Cultures were incubated at 37°C in a humidified 5% CO2 atmosphere and passaged every 5 days. Mycoplasma testing was routinely negative.

Paclitaxel. Paclitaxel was a gift from Bristol-Myers/Squibb. For all experiments, a concentrated paclitaxel solution (10 mM in DMSO, stored at 4°C) was diluted in medium to the desired concentration. In each experiment, control cells were exposed to a DMSO concentration equivalent to the highest DMSO concentration present in the paclitaxel-treated cells.

Growth Inhibition Assay. Exponentially growing cells were plated in triplicate at 3-5 × 10^4 cells/cm² in 24-well plates. After attachment, medium was changed, and cells were incubated in the presence or absence of at least eight paclitaxel concentrations. If drug exposure time was less than 120 h, cells were washed three times with medium (≥30-fold drug dilution with each wash) to ensure that the residual extracellular drug concentration was below that which would have effects over the total time of the experiment. After 120 h, the cells were detached by trypsinization and counted using a Coulter counter. IC₅₀ values were determined from plots of the percentage of the control cell number versus the logarithm of the drug concentration. All experiments were carried out at 37°C.
least twice, and values reported are means ± SDs of individual determinations from all experiments.

Assessment of Morphology. Exponentially growing MDA-MB-468 cells were incubated in the presence or absence of 100 nm paclitaxel for 3 or 24 h. Cells were then washed and incubated in drug-free medium for the additional periods of time before fixation in methanol. Fixed cells were stained with 0.1 mg/ml Hoechst dye no. 33342 (Sigma Chemical Co., St. Louis, MO) and visualized by fluorescence microscopy using a Zeiss Axioskop microscope (Zeiss, Hanover, MD) with filter set 48/79/02.

DNA Fragmentation Assays. Exponentially growing cells were plated at 3–5 × 10⁵ cells/cm². After attachment, the medium was changed, and cells were incubated in the presence or absence of paclitaxel for the desired exposure time. If the drug exposure time was shorter than the time to cell harvest, cells were washed and incubated in drug-free medium for the remainder of the total incubation time. At harvest, medium and trypsinized cells were combined and cells pelleted by centrifugation. For analysis of oligonucleosomal DNA fragmentation, DNA was isolated as previously described (6). Equivalent amounts of DNA (15–20 μg) were loaded into wells of a 1.6% agarose gel and electrophoresed in Tris-borate EDTA. For analysis of high-molecular-weight DNA fragmentation, the cell pellet was resuspended in 1% low-melting-point agarose and cast in a plug mold. After digestion with protease K and RNase A, plugs were loaded into wells of a 0.8% agarose gel and electrophoresed 12–14 h at 6 V/cm in 0.5X Tris-borate EDTA (1X Tris-borate EDTA is 89 mM Tris, 89 mM boric acid, and 2 mM EDTA) using program 6 of a PPI-200 field inversion timer (M. J. Research, Inc., Watertown, MA) to provide a linear time ramp from 0.3 to 30 s in the forward direction and a forward:reverse ratio of 3.

Quantitation of DNA Fragmentation. Experiments were carried out as stated above for analysis of high-molecular-weight fragmentation, with the following modifications. Exponentially growing cells were labeled with [methyl-14C]thymidine (0.05 mCi/ml medium, Amersham Life Science, Arlington Heights, IL) for 24 h before plating for DNA fragmentation assays. Throughout the cell harvest and processing of the agarose plugs, aliquots were removed and counted in the presence of fluor (Bio-Safe II, Research Products International, Mount Prospect, IL) to determine the percentage of total radioactivity in the medium and the washes. Gels were photographed with UV illumination and then dried onto DE81 paper on a vacuum drier. Fragmentation, as indicated by migration of DNA out of the wells, was quantitated by integration of signals using a phosphorimager (Molecular Dynamics, Sunnyvale, CA), and that percentage was added to the percentage of fragmentation determined from the medium and washes to assess the total percentage of DNA fragmentation.

Assessment of Apoptosis-related Proteolytic Cleavage. Cleavage of nuclear proteins was examined as described previously (11, 12, and references therein). In brief, samples containing 50 μg of protein from paclitaxel-treated cells were subjected to SDS-PAGE, transferred to nitrocellulose, and probed with the following antibodies: C-2-10, a murine monoclonal antibody against PARP3 (12); a chicken antiserum raised against mamalian lamin B1 (11); and a chicken antiserum raised against the abundant nucleolar protein B23 (11, 12).

RNA Isolation and Northern Blot Analysis. After incubation with paclitaxel or vehicle control, adherent and nonadherent cells were combined, and total cellular RNA was isolated using the urea-lithium chloride method of Auffray and Rougeon (13). Northern blot analysis was performed as described previously (6, 11). Quantitation of signals on autoradiographs was carried out using a phosphorimager, and results were normalized by expressing the units obtained for a specific transcript relative to the units obtained for the 18s ribosome transcript.

RESULTS

Inhibition of MDA-MB-468 Cell Growth by Paclitaxel. The effect of paclitaxel on MDA-MB-468 human breast cancer cells was examined. Initial experiments examined cell number 120 h after the start of 3-, 24-, or 120-h paclitaxel exposure (Fig. 1). These exposure times were chosen to model clinically used treatment times of 3, 24, and ≥96 h. Cells exposed to paclitaxel for only 3 h demonstrated concentration-dependent growth inhibition at ≥10 nm paclitaxel and an IC50 of 17 nm. Increasing the exposure time to 24 h resulted in increased sensitivity of MDA-MB-468 cells to paclitaxel, with concentration-dependent growth inhibition occurring at ≥1 nm paclitaxel and an IC50 of 2.6 nm. It is interesting to note that further increasing the paclitaxel exposure time had little effect, as the concentration dependence and the IC50 (1.8 nm) resulting from 120-h paclitaxel exposure were similar to those resulting from 24-h exposure. Ongoing clinical studies are addressing the optimal duration of paclitaxel treatment in breast cancer with particular interest in durations of 3 and 24 h. Pharmacokinetic studies during Phase I and II clinical trials have demonstrated peak plasma levels of 2.5–4.3 μM and 0.2–0.9 μM paclitaxel during 3- and 24-h infusions, respectively, and a steady-state plasma level of 0.05–0.09 μM paclitaxel during 96-h infusions (7, 9, 14). Therefore, 3- or 24-h exposure times and clinically achievable paclitaxel concentrations (0.1 nm to 1 μM) were used for all subsequent studies.

Induction of Programmed Cell Death by Paclitaxel. Characteristic morphological changes are associated with programmed cell death. These morphological changes include chromatin aggregation, nuclear condensation, and cellular fragmentation into apoptotic bodies (3, 4). MDA-MB-468 cells exposed to 100 nm paclitaxel for 3 h were assessed for these changes at times ranging from 6 to 48 h after initial drug exposure (Fig. 2). Chromatin aggregation and nuclear condensation were observed in a small number of the treated cells by 6 h (not shown), and the number of cells exhibiting these changes increased at 12 and 24 h (Fig. 2, B and C). Fragmentation of the cell into membrane-bound apoptotic bodies was also detected at 24 h and was markedly increased by 48 h (Fig. 2D). Cells exposed to paclitaxel for 24 h showed the same progression of morphological

3 The abbreviations used are: PARP, poly(ADP-ribose) polymerase; EGF, epidermal growth factor.
changes, but the time course was accelerated, with a larger number of apoptotic bodies present by 24 h (not shown). Untreated control cells fixed at each time point showed no evidence of these apoptosis-associated morphological changes, and mitotic figures were observed through the 48-h time point (Fig. 2A).

These morphological changes are frequently accompanied by fragmentation of genomic DNA into oligonucleosomal fragments (15). Paclitaxel exposure for 3 h resulted in time- and concentration-dependent oligonucleosomal DNA fragmentation characteristic of programmed cell death (Fig. 3A). Fragmentation induced by 3-h exposure to paclitaxel concentrations \( \geq 100 \, \text{nm} \) was detected at 24 h (Fig. 3A, Lanes 4 and 5), whereas that resulting from 10 nm paclitaxel was not detectable until 48 h after initial drug exposure (Fig. 3A, Lane 7). No oligonucleosomal DNA fragmentation was detected in DNA isolated from untreated controls at either 24 or 48 h (Fig. 3A, Lanes 2 and 6).

In additional experiments, paclitaxel exposure for 24 h at concentrations \( \geq 10 \, \text{nm} \) induced oligonucleosomal DNA fragmentation that was detectable at \( \approx 24 \, \text{h} \).

In some cell types undergoing programmed cell death, formation of oligonucleosomal DNA fragments appears to be preceded by two distinct events: (a) cleavage of DNA to domain-sized (\( \geq 50 \, \text{kb} \)) fragments (16); and (b) selective cleavage of several nuclear proteins by proteinases related to interleukin-1-\( \beta \) converting enzyme (12, 17). As one or both of these steps might play a role in commitment to programmed cell death (16, 17), the timing of both of these processes in paclitaxel-induced programmed cell death was examined.

Paclitaxel treatment of MDA-MB-468 cells resulted in a time- and concentration-dependent pattern of high-molecular-weight DNA fragmentation. Exposure to 100 nm paclitaxel for 3 or 24 h resulted in DNA fragments of \( \approx 50-150 \, \text{kb} \) detectable at 24, 48, and 72 h, but not at 6 h after initial drug exposure (Fig. 3B). Assay at 24 h indicated that paclitaxel concentrations \( \geq 10 \, \text{nm} \) were sufficient to induce DNA fragmentation even if the duration of drug exposure was only 3 h (not shown).

Examination of programmed cell death-related cleavage of nuclear proteins revealed a similar picture. Cleavage of the \( M_f \), 116,000 repair-related protein, PARP, to a \( M_f \) 85,000 fragment (12, 17) was detectable at \( \approx 24 \, \text{h} \) in cells exposed to 100 nm paclitaxel for 3 or 24 h (Fig. 3C, Lanes 3–5 and 7–9, respectively), whereas no cleavage of PARP was detectable at 6 h (Fig. 3C, Lanes 2 and 6). When cells were separated into adherent (not yet apoptotic) and nonadherent (apoptotic) cell populations as described previously (6, 11), PARP cleavage was detected exclusively in the nonadherent population (Fig. 3C, Lanes 11 and 14). No PARP cleavage was detectable in vehicle-treated control cells through 72 h (Fig. 3C, Lanes 16–19). Examination of lamina B\(_1\), an abundant nuclear envelope polypeptide that is cleaved to a \( M_f \) ~45,000 fragment during the course of programmed cell death (17 and references therein), revealed that cleavage of lamina B\(_1\) paralleled cleavage of PARP (Fig. 3C). In contrast, the abundant nucleolar protein B23 was not cleaved at any of the time points tested (Fig. 3C), an observation that is consistent with previous claims that the proteolytic process is selective for certain nuclear proteins (12, 17).

**Effects of Paclitaxel on Expression of Early-Response Genes.** In view of previous observations that different effectors of programmed cell death can have different effects on the expression of early-response genes (6, 11), the effects of paclitaxel on c-jun and c-myc expression in MDA-MB-468 cells were determined. A 2-fold increase in c-jun mRNA expression was observed 15 min after exposure to 100 nm paclitaxel. Expression of c-jun peaked at 2.4-fold induction at 30 min and returned to baseline levels at 1 h (Fig. 3D). Expression of c-myc did not change significantly during the 48-h period (Fig. 3D).

**Quantitation of Programmed Cell Death Induced by Paclitaxel.** The results described above provide clear evidence that 3- or 24-h paclitaxel exposure activates programmed cell death.
death pathway(s) in MDA-MB-468 cells. To assess whether the extent of programmed cell death resulting from 3-h versus 24-h paclitaxel exposures was comparable, DNA fragmentation and cell number were quantitated (Fig. 4). At all times >24 h, DNA fragmentation of treated cells was greater than that of untreated cells (Fig. 4A). Moreover, the amount of DNA fragmentation resulting from a 24-h exposure to 100 nM paclitaxel was greater than that resulting from a 3-h exposure (Fig. 4A). When the effects of 24-h versus 3-h paclitaxel were evaluated by comparing DNA fragmentation 72 h after the initiation of treatments that involved similar concentration × time parameters (“area under the curve”), greater induction of programmed cell death by the 24-h treatment remained evident. For example, treatment with 10 nM paclitaxel for 24 h (240 nM·h) provided lower exposure than 100 nM paclitaxel for 3 h (300 nM·h), yet the DNA fragmentation with the 24-h treatment was 1.5-fold higher (63.5 ± 8.6% versus 42.9 ± 3.1%; Fig. 4B). Similarly, treatment with 100 nM paclitaxel for 24 h (2400 nM·h) involved lower exposure than 1 μM for 3 h (3000 nM·h), yet the DNA fragmentation 72 h after the initiation of the 24-h treatment was greater than that 72 h after the initiation of the 3-h treatment (76.6 ± 6.9 versus 53.9 ± 9.9%; Fig. 4B). Indeed, 24-h exposure to 10 nM paclitaxel produced more DNA fragmentation than 3-h exposure to 1 μM (63.5 ± 8.6% versus 53.9 ± 9.9%), although the area under the curve with the 24-h treatment (240 nM·h) was only 8% of the exposure in the 3-h treatment (3000 nM·h). These comparisons indicate that lower concentrations of paclitaxel are required to achieve equal or greater DNA fragmentation when the longer exposure time of 24 h is used.

The enhanced effectiveness of the prolonged paclitaxel exposure was also evident when cell numbers were examined. For example, 10 nM paclitaxel for 24 h (240 nM·h) and 100 nM for 3 h (300 nM·h) resulted in 74% and 59% decreases in cell number, respectively. Likewise, 100 nM paclitaxel for 24 h (2400 nM·h) and 1 μM paclitaxel for 3 h (3000 nM·h) resulted in 83% and 70% decreases in cell number, respectively. Although the area under the curve was smaller in each case, the cell decrease was more profound.

Fig. 2. Fluorescent micrographs of MDA-MB-468 cells incubated in the presence or absence of 100 nM paclitaxel. MDA-MB-468 cells were exposed to 100 nM paclitaxel (B-D) or vehicle control (A) for 3 h and then incubated in drug-free medium until fixation and staining with Hoechst’s dye no. 33342 at 12 (B), 24 (C), or 48 (A and D) h.
with the 24-h treatment. Indeed, decreases in cell number were similar after treatment with 10 nM paclitaxel for 24 h (74%) and 1 μM paclitaxel for 3 h (70%), although the area under the curve of the 24 h treatment was less than 10% of that obtained with the 3-h treatment (240 nm · h versus 3000 nm · h). There are also additional indications that the longer exposure time is more toxic even at lower drug concentrations. After the 3-h exposure to 100 nM paclitaxel, an increase in cell number over the number at the time of initial drug exposure was observed at times >24 h, and cell number at 96 h was 2.4 times greater than the original number (Fig. 4C). Even at the highest paclitaxel concentration tested (1 μM), the 3-h exposure resulted in a net increase in cell number over that at the time of initial drug exposure (Fig. 4D). In contrast,

Fig. 3 Paclitaxel induction of programmed cell death in MDA-MB-468 cells. A, induction of oligonucleosomal-sized DNA fragmentation by paclitaxel. Cells were exposed to paclitaxel (P) or vehicle (U) for 3 h and then incubated in drug-free medium until 24 or 48 h after initial drug exposure. Isolated DNA was analyzed by gel electrophoresis. Lane 1, 123-bp ladder marker; Lane 2, vehicle control, 24 h; Lanes 3–5, 10 nM, 100 nM, and 1 μM paclitaxel, respectively, 24 h; Lane 6, vehicle control, 48 h; Lanes 7 and 8, 10 nM and 1 μM paclitaxel, respectively, 48 h. B, paclitaxel induction of high-molecular-weight DNA fragmentation. MDA-MB-468 cells were incubated in paclitaxel or vehicle for up to 24 h and incubated in drug-free medium for any additional period before harvest for field inversion gel electrophoresis. Lane 1, λ HindIII molecular weight marker; Lane 2, vehicle control, 6 h; Lane 3, 100 nM paclitaxel, 6 h; Lane 4, vehicle control, 24 h; Lanes 5–9, 0.1 nM, 1 nM, 10 nM, 100 nM, and 1 μM paclitaxel, respectively, 24 h; Lane 10, vehicle control, 48 h; Lane 11, 100 nM paclitaxel, 48 h; Lane 12, untreated control, 72 h; Lane 13, 100 nM paclitaxel, 72 h. C, paclitaxel induction of proteolytic cleavage. MDA-MB-468 cells were exposed to 100 nM paclitaxel for 3 or 24 h. Cleavage of PARP, lamin B1, and B23 was assessed at 3, 6, 24, 48, and 72 h after initial drug exposure. Lanes 1–5, 24-h paclitaxel exposure, harvest at 3, 6, 24, 48, and 72 h, respectively; Lanes 6–9, 3-h paclitaxel exposure, harvest at 6, 24, 48, and 72 h, respectively; Lanes 10–12, 24-h paclitaxel exposure with immediate harvest for total cell population, nonadherent cells, and adherent cells, respectively; Lanes 13–15, 24-h paclitaxel exposure with harvest at 48 h for total cell population, nonadherent cells, and adherent cells, respectively; Lanes 16–18, 24-h paclitaxel exposure with harvest at 48 h for total cell population, nonadherent cells, and adherent cells, respectively. D, Northern blot analysis of RNA isolated from MDA-MB-468 cells exposed to paclitaxel for 3 h. U, vehicle-treated control cells; P, paclitaxel-treated cells; subscripts, time of harvest (h).
Fig. 4 Quantitation of DNA fragmentation and cell numbers after exposure of MDA-MB-468 cells to paclitaxel. A, time dependence of DNA fragmentation resulting from 3- or 24-h exposure to 100 nM paclitaxel. B, concentration dependence of DNA fragmentation assayed 72 h after initial exposure of cells to paclitaxel for 3 or 24 h. At 72 h, there was 23% DNA fragmentation in vehicle-treated control cells. DNA fragmentation resulting from paclitaxel was quantitated as described in "Materials and Methods." Values reported in A and B are the means of three or more individual determinations from at least two separate experiments; bars, SD. C, time dependence of cell number after 3- or 24-h exposure to 100 nM paclitaxel. D, concentration dependence of cell number assayed 72 h after initial exposure of cells to paclitaxel for 3 or 24 h. Cell number at the time of initial drug exposure was $2.5 \times 10^4$ cells/cm$^2$. Values reported are from a representative experiment and represent means of at least three individual determinations for C and averages of two individual determinations for D; bars, SD.

after 24-h paclitaxel (100 nM) exposure, the cell numbers at each assay time remained at or below the original number (Fig. 4C), and a net decrease in cell number was evident after 24-h exposure to concentrations ≥100 nM (Fig. 4D).

**Effects of Paclitaxel in Other Breast Cancer Cell Lines.** To determine whether paclitaxel induces programmed cell death in other human breast cancer cell lines, MDA-MB-231, HS578t, and MCF-7 breast cancer cells were exposed to 100 nM paclitaxel for 24 h and monitored over a 72-h period. Morphological changes and high-molecular-weight DNA fragmentation were observed in each of these cell lines (data not shown), indicating induction of programmed cell death by paclitaxel. Quantitation of the amount of DNA fragmentation indicated that 24-h paclitaxel exposure resulted in rapid and extensive DNA fragmentation in HS578t cells, in as much as there was 53% DNA fragmentation by 24 h and 96% at 72 h after initial drug treatment (Fig. 5A). Although the effects were not as rapid in the other cell lines, at 72 h, DNA fragmentation was 40% and 21%, respectively, for MDA-MB-231 and MCF-7 cells, levels significantly greater than those observed in the untreated control cells (Fig. 5A). Examination of cell numbers present at each harvest time indicated that 24-h exposure to 100 nM paclitaxel prevented an increase in total cell number of MDA-MB-231 and MCF-7 cell populations over the 72 h observed, and resulted in a
Fig. 5  Quantitation of DNA fragmentation and cell numbers after exposure of MDA-MB-231, HS578t, and MCF-7 cells to paclitaxel. A. time dependence of DNA fragmentation resulting from 24-h exposure to 100 nM paclitaxel. B. time dependence of cell number after 24-h exposure to 100 nM paclitaxel. Values reported in A and B are the average of two individual determinations.

DISCUSSION

Paclitaxel has substantial clinical activity against a variety of malignancies, including breast cancer. The present study shows that exposure to clinically attainable paclitaxel concentrations (7, 9, 14) for 24 h induces programmed cell death in all four breast cancer cell lines tested. In addition, exposure to clinically attainable paclitaxel concentrations (7, 9, 14) for 3 h is sufficient to activate programmed cell death pathway(s) in MDA-MB-468 cells. Even this brief exposure resulted in characteristic morphological changes, DNA fragmentation to both high-molecular-weight and oligonucleosomal-sized fragments, and proteolytic cleavage of PARP and lamin B1, although the changes were not evident until some hours later.

Paclitaxel effects in MDA-MB-468 cells differ from those that result from activation of the cell death program by fluoropyrimidines or EGF (6, 11). Quantitation of DNA fragmentation indicated that 24-h paclitaxel exposure (100 nM) results in ~2-fold greater DNA fragmentation at 48 h than does continuous exposure to either EGF (10 ng/ml) or 5-fluoro-2'-deoxyuridine (100 μM), although similar amounts of fragmentation are observed at 72 and 96 h. Exposure to paclitaxel or EGF, but not to 5-fluoro-2'-deoxyuridine, led to rapid induction of the early response gene c-jun. However, the peak level of induction after paclitaxel was 2.4-fold compared with 16-fold induction of c-jun after EGF. Neither paclitaxel nor 5-fluoro-2'-deoxyuridine induced c-myc expression, whereas EGF resulted in an 11-fold increase of c-myc expression. The variety of cellular responses to different effectors of programmed cell death within the same cell type suggests that these responses are secondary to the as-yet-unidentified critical event(s) that actually trigger activation of the cell death pathway.

Paclitaxel effects in the breast cancer cells during activation of programmed cell death also differ from those reported in other cell types. In HL-60 leukemia cells, paclitaxel-induced programmed cell death is accompanied by a decrease in c-myc expression without a change in c-jun expression at ≥24 h. The possibility that a transient induction could have occurred at earlier times was not examined (18).

Finally, it is currently unclear whether the variety of clinical protocols that include infusion times of 3, 24, or 96 h, currently used for paclitaxel, are equally efficacious (7, 8, 19). It has been reported that 4- and 12-h paclitaxel exposures are required to induce programmed cell death in HL-60 cells (18) and OV2008 ovarian carcinoma cells (20), respectively. Our studies provide clear evidence that paclitaxel exposure for either 3 or 24 h can increase the rate of cell death in MDA-MB-468 breast cancer cells (Figs. 2 and 3), but the two treatments differ in their ability to alter the overall growth of the MDA-MB-468 cell population. After 3-h paclitaxel exposure, the overall cell number continued to increase despite evidence of programmed cell death occurring in some cells (Fig. 4, C and D). This indicates that a portion of the cell population was still actively proliferating, and the rate of cell proliferation still exceeded the rate of cell death. In contrast, after exposure to paclitaxel for 24 h, the rate of cell death exceeded the rate of cell proliferation, because the population cell number remained at or below the original number at all times, and no net increase in cell number occurred (Fig. 4, C and D). These results demonstrate quantitatively that 24-h paclitaxel exposure was more effective than 3-h exposure at inducing programmed cell death and stopping cell proliferation, even when treatments with similar areas under the...
curve were compared. These *in vitro* studies cannot mimic the pharmacokinetic or pharmacodynamic situation encountered clinically. However, these results do support those clinical studies that suggest that longer paclitaxel infusion times are of greater benefit, and suggest an explanation at the cellular level for this observed outcome. Additionally, if the same situation exists *in vivo*, these results suggest that at least part of the lack of response seen clinically to 3-h paclitaxel infusions may not result from inherent resistance of the cells to this agent, but from a lack of adequate time for a large portion of the cell population to initiate a response.

**ACKNOWLEDGMENTS**

We thank Drs. John Isaacs and Lars Cisek for helpful discussions.

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Paclitaxel induces programmed cell death in MDA-MB-468 human breast cancer cells.
