

Resistance to Paclitaxel Is Proportional to Cellular Total Antioxidant Capacity

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Abstract

Paclitaxel, one of the most commonly prescribed chemotherapeutic agents, is active against a wide spectrum of human cancer. The mechanism of its cytotoxicity, however, remains controversial. Our results indicate that paclitaxel treatment increases levels of superoxide, hydrogen peroxide, nitric oxide (NO), oxidative DNA adducts, G₂-M arrest, and cells with fragmented nuclei. Antioxidants pyruvate and selenium, the NO synthase inhibitor N^ω-nitro-L-arginine methyl ester, and the NO scavenger manganese (III) 2-(4-carboxyphenyl)-4,4,5,5-tetramethyl-imidazole-1-oxyl-3-oxide all decreased paclitaxel-mediated DNA damage and sub-G₁ cells. In contrast, the glutamylcysteine synthase inhibitor buthionine sulfoximine (BSO) and the superoxide dismutase (SOD) inhibitor 2-methoxyestradiol (2-ME) increased the sub-G₁ fraction in paclitaxel-treated cells. These results suggest that reactive oxygen and nitrogen species are involved in paclitaxel cytotoxicity. This notion is further supported with the observation that concentrations of paclitaxel required to inhibit cell growth by 50% correlate with total antioxidant capacity. Moreover, agents such as arsenic trioxide (As₂O₃), BSO, 2-ME, PD98059, U0126 [mitogen-activated protein/extracellular signal-regulated kinase inhibitors], and LY294002 (phosphatidylinositol 3-kinase/Akt inhibitor), all of which decrease clonogenic survival, also decrease the total antioxidant capacity of paclitaxel-treated cells, regardless whether they are paclitaxel sensitive or paclitaxel resistant. These results suggest that paclitaxel chemosensitivity may be predicted by taking total antioxidant capacity measurements from clinical tumor samples. This, in turn, may then improve treatment outcomes by selecting out potentially responsive patients. (Cancer Res 2005; 65(18): 8455-60)

Introduction

Paclitaxel, originally isolated from *Taxus brevifolia* (pacific yew), is one of the most active chemotherapeutic agents against a wide panel of solid tumors including urothelial, breast, lung, and ovarian cancers (1, 2). The mechanism of paclitaxel cytotoxicity, however, remains controversial. Paclitaxel promotes the stable assembly of microtubules from α - and β -tubulin heterodimers and inhibits their de-polymerization (3). Thus, the antitumor effects of this drug

may result from interference with the normal function of microtubules and from blocking of cell cycle progression in late G₂-M phases (4). Paclitaxel-induced apoptosis in hepatoma cells is mediated through G₂-M arrest and DNA fragmentation (5). Cells with a defective G₁ checkpoint and with an increased percentage of G₂-M fractions were found to have increased sensitivity to paclitaxel (6–8). However, the observation that in some cell lines, pulsed paclitaxel exposures causes apoptosis but not G₂-M arrest suggests that paclitaxel-induced apoptosis may occur without a prior G₂-M arrest (9). Moreover, paclitaxel has been shown to induce apoptosis in G₁ and S stages, but induce both apoptosis and necrosis in G₂-M phase (10).

Paclitaxel has been reported to induce the formation of reactive oxygen species (ROS) and alter mitochondrial membrane permeability (11). Reduction of ROS by catalase or ascorbic acid treatment, however, does not correlate with the reduction of cytotoxicity in the human herpes virus 8-related tumor cell line BCBL-1, suggesting that oxidative stress is only partially involved in paclitaxel cytotoxicity (12). Moreover, treatment of the human T-cell lymphoblastic leukemia cell line CCRF-HSB-2 with the antioxidant N-acetyl-L-cysteine showed inhibition of paclitaxel-induced ROS production but did not prevent paclitaxel-induced apoptosis, indicating that paclitaxel-induced apoptosis in these cells is ROS independent (13). In murine bladder tumor MBT-2 cells, paclitaxel has also been shown to activate a macrophage-mediated antitumor mechanism through a nitric oxide (NO)-dependent pathway (14). Cotreating the human myeloid leukemia cell line HL-60 with paclitaxel and the NO-generating agent S-nitrosoglutathione decreases the accumulation of G₂-M fractions, suggesting that NO prevents paclitaxel-treated cells from entering the G₂-M phase (15).

The current study reveals our investigation into the role(s) of ROS and reactive nitrogen species in paclitaxel toxicity. Results support our hypothesis that ROS and reactive nitrogen species are involved in paclitaxel-induced apoptosis. We further show that in a wide panel of human cancer cell lines, cellular total antioxidant capacity is a critical determinant of cellular sensitivity to paclitaxel.

Materials and Methods

Cells. Cell lines MCF-7 and HCC1937 were cultured in DMEM. H460, H1299, H1355, SC-M1, HR, NTUB1 (16), and BFTC905 (17) were cultured in RPMI 1640. SV-HUC-1, 293, and T24 were cultured in F-12 medium; BEAS-2B was cultured in LHC-9 medium (BioSource International, Inc., Camarillo, CA); T24/A (18) was cultured in RPMI 1640 containing 0.4 μ mol/L doxorubicin. NTUB1/P and NTUB1/T were maintained in RPMI 1640 containing 14 μ mol/L cisplatin and 5 nmol/L paclitaxel, respectively (19). All growth media were supplemented with 10% FCS, penicillin (100 units/mL), streptomycin (100 μ g/mL), and 0.03% glutamine. Cultures were maintained at 37°C in a water-saturated atmosphere containing 5% CO₂.

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BEAS-2B is an immortalized human bronchial epithelial cell line and MCF-7 and HCC1937 are breast cancer cell lines. H460, H1299, and H1355 are lung cancer cell lines; SC-M1 and HR are gastric cancer cell lines. The 293 cell line is derived from adenovirus-transformed human embryonic kidney epithelial cells. T24, BFTC905, and NTUB1 are human bladder urothelial carcinoma cell lines; SV-HUC-1 is an SV40 large T-transformed human urothelial cell line; T24/A is a doxorubicin-resistant subline to T24. NTUB1/P and NTUB1/T are cisplatin- and paclitaxel-resistant sublines to NTUB1, respectively.

Measurement of O_2^- , H_2O_2 , nitric oxide, and total antioxidant capacity. Superoxide production was measured using the chemiluminescence probe L-012 as previously described (20). Cellular levels of H_2O_2 were measured with the aid of the fluorogenic probe Amplex Red (21). NO production was measured by detecting nitrite released in culture medium using 2,3-diaminonaphthalene (22). For determining cellular total antioxidant capacity, posttreatment cells (1×10^6) were washed with PBS and suspended in 200 μ L of ice-cold lysis buffer [10 mmol/L Tris-HCl (pH 7.4), 1 mmol/L EDTA, 0.5 mmol/L phenylmethylsulfonyl fluoride, 0.5 μ g/mL leupeptin, 0.5 μ g/mL pepstatin] and sonicated for 3 minutes with a 9-second pulse and 1 second off. The lysate was centrifuged at 10,000 rpm for 10 minutes, and protein concentration of the supernatant fraction was determined with a Bio-Rad Protein Assay Kit (Hercules, CA). Bovine serum albumin was used as a standard. The Trolox equivalent antioxidant activity was measured by assessing the ability of hydrogen-donating antioxidants to scavenge the radical cation generated by 2,2'-azinobis(3-ethylbenzothiazoline)-6-sulfonic acid (23).

Measurement of DNA damage. The comet assay, involving an incubation with endonuclease III and formamidopyrimidine-DNA glycosylase, was carried out as described (24). Migration of DNA from the nucleus in each cell was measured with Comet Assay III software.⁴

Measurement of G_2 -M and sub- G_1 fractions. Cells seeded for overnight incubation were treated with paclitaxel for 12 hours to measure G_2 -M fractions. For sub- G_1 fraction measurements, cells were treated with paclitaxel for 48 hours. The DNA histogram was measured by an EPICS XL-MCL flow cytometer (Beckman-Coulter) with excitation at 488 nm and emission at 620 nm, as previously described (25).

Measurement of nuclear fragmentation. Cells seeded for overnight incubation were treated with paclitaxel for 48 hours. The nuclear integrity of 500 cells was examined for each treatment as previously described (25).

Measurement of cell growth inhibition. The cytotoxic effect of paclitaxel was measured using standard 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assay.

Colony formation assay. Cells were seeded into 60 mm Petri dishes and left in the incubator for 6 hours to allow cell attachment. The number of cells seeded per dish was varied so that about 100 colonies were counted after a 12-day incubation period. For each treatment, three dishes of cells were plated. The cells were treated with different drugs, with or without paclitaxel, for 12 days. After 12 days, colonies were fixed for 10 minutes in 100% methanol and stained with a 10% Giemsa solution for 10 minutes. Colonies containing over 50 cells were counted. Percent colony formation was calculated by assigning untreated cultures as 100%. The percent colony formation of treated cells was calculated by using the following formula: percent colony formation of treated cells = (colony formation of treated cells / colony formation of untreated cells) \times 100.

Statistical methods. All experiments were done independently at least thrice. The mean of each independent experiment was collected and data are expressed as mean \pm SD. Statistical comparisons were made using Student's *t* test. Pearson's correlation tests were carried out to measure the relationship between cellular IC₅₀ and total antioxidant capacity. All tests were two sided with $P < 0.001$ as statistically significant.

Results

Paclitaxel induces reactive oxygen species in T24 cells. Treating T24 cells with paclitaxel significantly increased fluores-

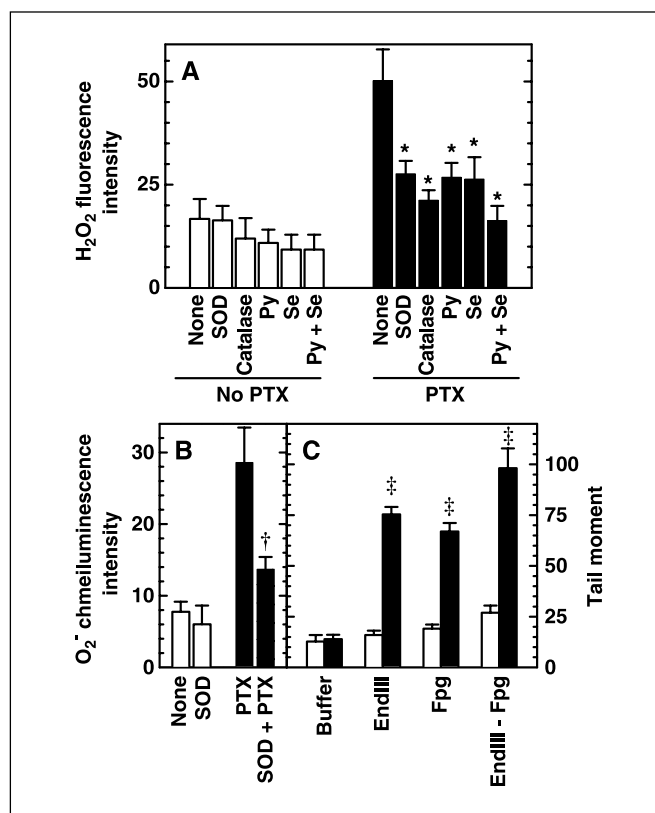


Figure 1. Treatment with paclitaxel increased levels of ROS and oxidative DNA damage in T24 cells. **A**, cells were treated for 2 hours with 100 milliunits SOD, 200 milliunits catalase, 2 μ mol/L pyruvate (Py), and 2 μ mol/L selenium (Se) with or without 0.04 μ mol/L paclitaxel (PTX). Cellular levels of H_2O_2 were measured with the fluorescence probe Amplex Red. *, $P < 0.001$, paclitaxel versus paclitaxel plus antioxidant. **B**, cells were treated for 2 hours with 100 milliunits SOD and/or 0.04 μ mol/L paclitaxel. The chemiluminescent intensity of L-012, reflecting the superoxide level, was then measured. †, $P < 0.001$, paclitaxel versus paclitaxel plus SOD. **C**, cells were treated (solid columns) or untreated (open columns) with 0.02 μ mol/L paclitaxel for 6 hours and the level of DNA strand breaks was measured using the comet assay with or without endonuclease III (EndIII) and/or formamidopyrimidine-DNA glycosylase (Fpg) digestion. †, $P < 0.001$, paclitaxel without versus paclitaxel with enzyme digestion. Columns, mean of three experiments; bars, SD.

cence intensity of Amplex Red. This effect is reduced by antioxidants, superoxide dismutase (SOD), catalase, pyruvate, and selenium (Fig. 1A). These results indicate that paclitaxel treatment increases intracellular H_2O_2 levels. Paclitaxel treatment also increases chemiluminescent intensity of L-012, a probe used for measuring O_2^- (Fig. 1B). Production of O_2^- is decreased in the presence of SOD. Treating T24 cells with paclitaxel did not induce any DNA strand break, as analyzed by the standard comet assay. However, large amounts of DNA strand breaks are generated by incubating paclitaxel-treated T24 cells with endonuclease III or formamidopyrimidine-DNA glycosylase because endonuclease III removes oxidized pyrimidines and formamidopyrimidine-DNA glycosylase removes oxidized purines (26). These results suggest paclitaxel induces oxidative DNA damages (Fig. 1C). Similarly, the extent of oxidative base-specific DNA strand breaks is significantly reduced in the presence of antioxidants pyruvate and/or selenium (Fig. 2A).

Paclitaxel induces G_2 -M arrest, nuclear fragmentation, and cell growth inhibition. Paclitaxel treatment induces significant accumulation of cells in the G_2 -M phase (Fig. 2B), increases the proportion of cells with fragmented nuclei (Fig. 2C), and inhibits cell

⁴ <http://www.perceptive.co.uk>.

growth (Fig. 2D). All of these paclitaxel-mediated cytotoxic effects are partially suppressed by pyruvate and/or selenium.

Paclitaxel induces nitric oxide production in T24 cells.

Treating T24 cells with paclitaxel increased NO production as evidenced by the increase of nitrite levels in the culture medium (Fig. 3A). NO production was also suppressed by the NO synthase inhibitor *N*^ω-nitro-L-arginine methyl ester (NAME) and the NO scavenger manganese (III) 2-(4-carboxyphenyl)-4,4,5,5-tetramethylimidazole-1-oxyl-3-oxide (c-PTIO). These NO modulators also partially salvaged the paclitaxel-mediated oxidative DNA damage as shown by the comet assay (Fig. 3B).

Antioxidants and nitric oxide modulators suppress paclitaxel toxicity in various cell lines. Results thus far suggest that H₂O₂, O₂⁻, and NO may all be involved in paclitaxel-induced cytotoxicity observed in T24 cells. To investigate whether or not this is a cell-specific phenomenon, we next examined the effect of antioxidants (pyruvate plus selenium) and NO modulators (NAME or c-PTIO), using DNA flow cytometry, on paclitaxel-induced sub-G₁ fraction accumulation in cell lines other than T24 cells, including two other human urothelial carcinoma cell lines (BFTC905 and NTUB1), one SV40-transformed human urothelial cell line (SV-HUC-1), one human lung epidermoid carcinoma cell line (H1355), and one human breast cancer cell line (MCF-7; Fig. 4). Results indicate that pyruvate plus selenium, NAME, and c-PTIO significantly decrease paclitaxel-induced sub-G₁ fraction accumulation in these cell types (Fig. 4A and B). Moreover, blocking glutathione synthesis with buthionine sulfoximine (BSO) or inhibiting SOD activity with 2-methoxyestradiol (2-ME) enhances paclitaxel cytotoxicity as evidenced by a significant enhancement in accumulation of sub-G₁ fractions

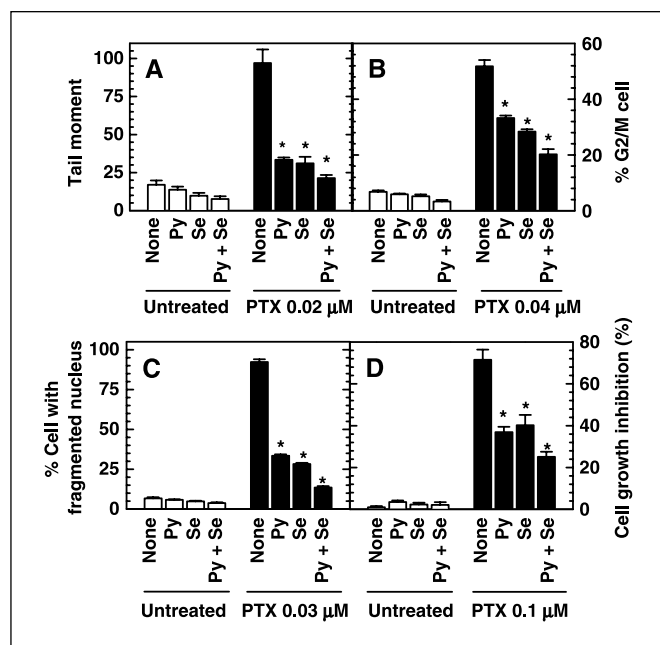


Figure 2. Antioxidants pyruvate and selenium suppressed paclitaxel-induced oxidative base-specific DNA strand breaks (A), G₂-M arrest (B), nuclear fragmentation (C), and cell growth inhibition (D) in T24 cells. Cells were treated for 6 hours (A), 12 hours (B), 48 hours (C), or 72 hours (D) with paclitaxel alone or in combination with 2 μmol/L pyruvate and/or 2 μmol/L selenium. The use of different concentrations of paclitaxel and different exposure time was intended to show substantial changes in each variable by antioxidants. *, *P* < 0.001, paclitaxel versus paclitaxel plus antioxidant(s). Columns, mean of three experiments; bars, SD.

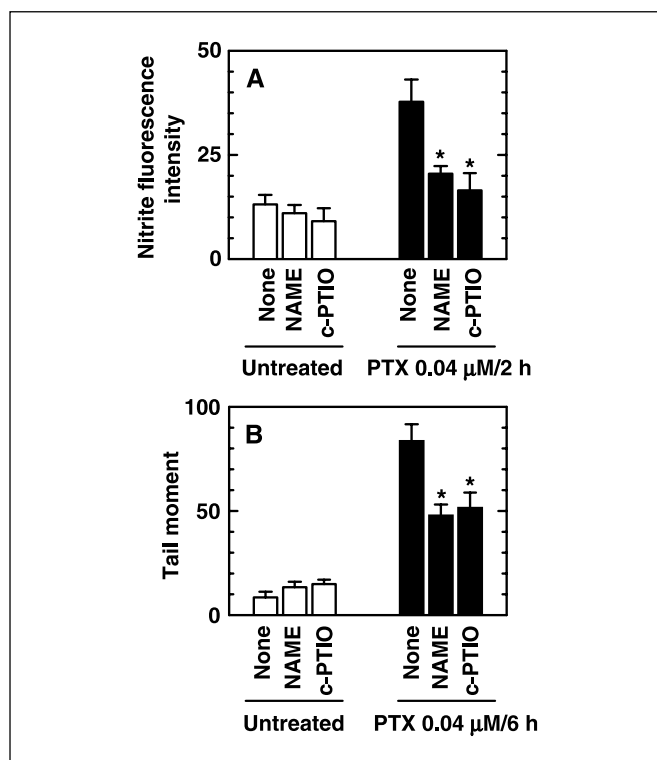


Figure 3. A, treatment with paclitaxel increased NO levels in T24 cells. Cells were treated for 2 hours with 100 μmol/L NAME and 100 μmol/L c-PTIO, with or without 0.04 μmol/L paclitaxel. Nitrite fluorescence intensity in the medium was then measured. B, NO modulators, NAME, and c-PTIO decreased paclitaxel-induced DNA damage. Cells were treated with NO modulators, with or without paclitaxel, for 6 hours. DNA damage was then measured by the comet assay with nuclear extract incubation. *, *P* < 0.001, paclitaxel versus paclitaxel plus NO modulator.

(Fig. 4C and D). These data indicate that in a wide spectrum of cell lines, ROS and NO are both involved in paclitaxel-induced cytotoxicity.

Total antioxidant capacity correlates to paclitaxel resistance. Because H₂O₂, O₂⁻, and NO were found to be involved in paclitaxel-induced cytotoxicity, we hypothesized that tumor cells with higher total antioxidant capacity would be more resistant to paclitaxel than those with lower total antioxidant capacity. To test this hypothesis, we measured total antioxidant capacity (Fig. 5A) and paclitaxel IC₅₀ (Fig. 5B) of 16 different cell lines. Results showed that total antioxidant capacity had a positive correlation with the paclitaxel IC₅₀ measured by the MTT assay (Pearson's correlation coefficient *r* = 0.90, *P* < 0.0001; Fig. 5D). We further confirmed these results by next performing a colony formation assay (Fig. 5D). Cell lines selected for the colony formation assay included MCF-7 (a highly paclitaxel-sensitive line), T24 (a paclitaxel-sensitive line), T24/A (a doxorubicin-resistant subline to T24), and NTUB1/P and NTUB1/T (cisplatin- and paclitaxel-resistant sublines to NTUB1, respectively). Data from the colony formation assay confirmed our previous results, indicating that total antioxidant capacity correlates very well with the paclitaxel IC₅₀ (Pearson's correlation coefficient *r* = 0.93, *P* = 0.024; Fig. 5D).

Agents that reduce paclitaxel resistance also decrease total antioxidant capacity. The above results suggest that the higher the paclitaxel IC₅₀ of tumor cells, the higher the total antioxidant capacity. We tested this hypothesis by examining whether the

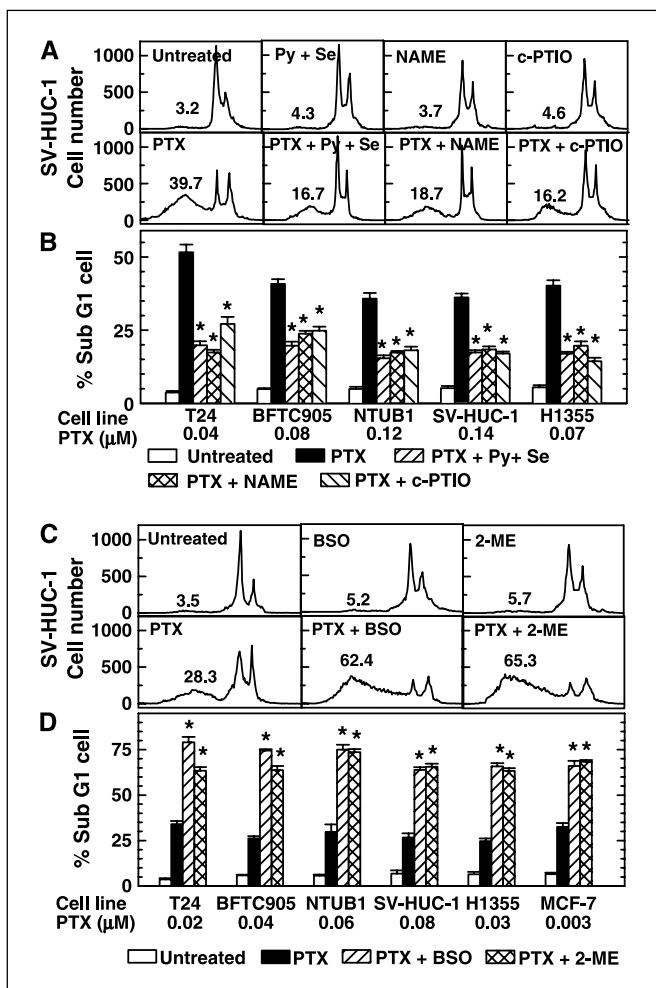


Figure 4. A and B, antioxidants and nitric oxide modulators decreased paclitaxel-induced apoptosis. C and D, BSO and 2-ME increased the apoptosis. Cells were treated with drugs for 48 hours. A and C, the percentage of cells with sub-G₁ DNA content is indicated. A and B, cells were treated with higher concentrations of paclitaxel than in C and D. This design was intended to give more room for demonstrating decreasing and increasing effects of the modulators. The sub-G₁ fractions were very low (<6%) in untreated cultures or cells treated with 2 $\mu\text{mol/L}$ pyruvate plus 2 $\mu\text{mol/L}$ selenium, 100 $\mu\text{mol/L}$ NAME, 100 $\mu\text{mol/L}$ c-PTIO, 50 $\mu\text{mol/L}$ BSO, or 10 $\mu\text{mol/L}$ 2-ME. *, $P < 0.001$, paclitaxel alone versus paclitaxel plus modulator.

agents that reduce paclitaxel resistance would also reduce cellular total antioxidant capacity. Results indicate that in MCF-7 (the most sensitive cell line) and NTUB1/T cells (the most resistant cell line), PD98059 [a mitogen-activated protein/extracellular signal-regulated kinase (MEK/ERK) inhibitor], U0126 (a MEK/ERK inhibitor), LY294002 (a phosphatidylinositol 3-kinase/Akt inhibitor), BSO, 2-ME, and As_2O_3 (an ROS-generating agent) all significantly reduce clonogenic survival (Fig. 6A and B) and decrease total cellular antioxidant capacity (Fig. 6C and D). Similar results were observed for other cell lines, including T24, T24/A, and NTUB1/P cells (data not shown).

Discussion

In this study, evidence has been collected that supports the notion that paclitaxel may exert its toxicity via elevation of intracellular O_2^- , H_2O_2 , and NO levels. This theory is confirmed by our data showing that (a) paclitaxel induced the production

of O_2^- , H_2O_2 and NO; (b) paclitaxel induced oxidative DNA damage; (c) agents that decreased H_2O_2 and NO production suppressed paclitaxel-induced DNA damage, G₂-M arrest, apoptosis, and cell growth inhibition; (d) inhibition of SOD or glutamylcysteine synthase increased paclitaxel-induced apoptosis; (e) cell lines with higher total antioxidant capacity were more resistant to paclitaxel cytotoxicity; and (f) agents that decreased clonogenic survival in paclitaxel-treated cells also decreased cellular total antioxidant capacity. Thus, paclitaxel chemoresistance correlates very well to intracellular antioxidant capacity.

Kong et al. (27) speculated that many chemotherapeutic agents exert their toxic effects on cancer cells by producing free radicals, leading to irreversible cell injury, and that overproduction of ROS in cancer cells may exhaust the capacity of SOD and other adaptive antioxidant defenses. This concept is consistent with our results showing that depletion of cellular antioxidant

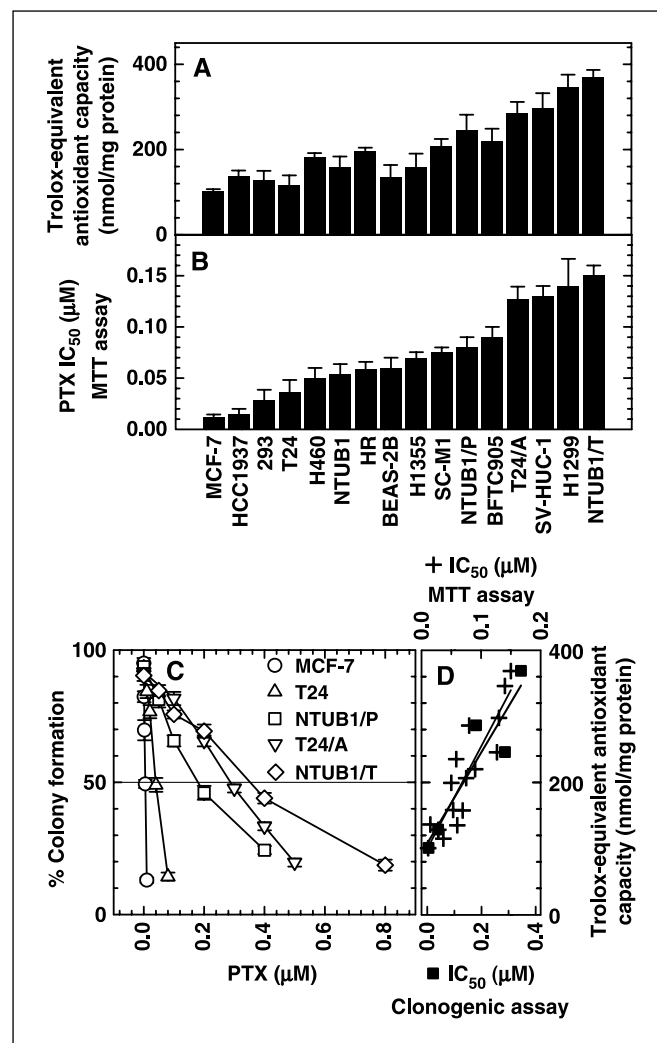
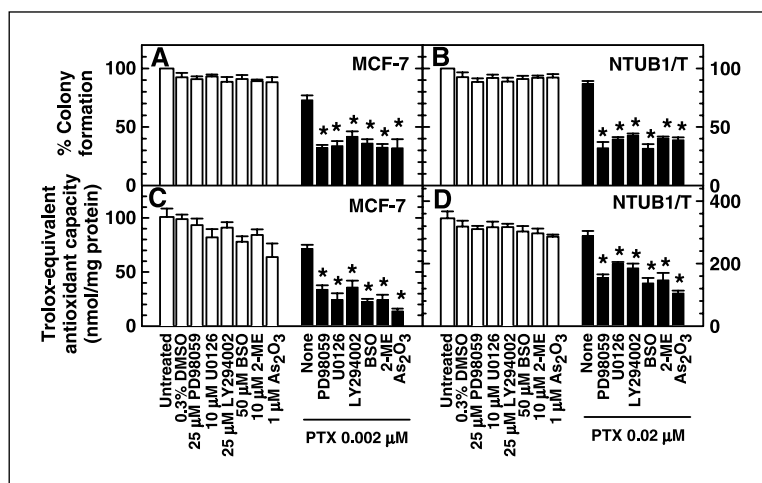


Figure 5. Resistance to paclitaxel positively correlated with cellular total antioxidant capacity. A, cells were seeded and incubated overnight; total antioxidant capacity was then measured. B, cells were treated with paclitaxel for 72 hours. The paclitaxel IC₅₀s were determined by the MTT method. C, cell growth inhibition was determined with the colony formation assay in five selected cells. Cultures were treated with various concentrations of paclitaxel for 12 days; colony numbers were then counted. D, total antioxidant capacities were plotted against the paclitaxel IC₅₀s from MTT assay (Pearson's correlation $r = 0.90$, $P < 0.0001$) and from the clonogenic assay (Pearson's correlation $r = 0.93$, $P < 0.024$).

Figure 6. PD98059, U0126, LY294002, BSO, 2-ME, and As_2O_3 decreased the cellular total antioxidant capacity and the clonogenic survival in paclitaxel-treated MCF-7 (paclitaxel-sensitive) and NTUB1/T (paclitaxel-resistant) cells. For total antioxidant capacity and the clonogenic assay, cells were treated with drugs for 24 hours and 12 days, respectively. *, $P < 0.001$, paclitaxel versus paclitaxel plus modulator.



capacity enhanced paclitaxel toxicity. Recently, it was reported that paclitaxel treatment activates the MEK/ERK and phosphatidylinositol 3-kinase/Akt signaling pathways (28). Inhibiting these pathways with PD98059, U0126, or LY294002 down-regulated paclitaxel-mediated survivin induction and enhanced cell death in MCF-7 cells. Our data further showed that inhibition of these pathways also enhanced paclitaxel-induced cell death in NTUB1/P, T24/A, and NTUB1/T cells, which were respectively about 25, 42, and 53 times more resistant to paclitaxel than the MCF-7 cells. Neither MEK inhibitors PD98059 and U0126 nor phosphatidylinositol 3-kinase inhibitor LY294002 alone decreased the intracellular total antioxidant capacity. However, these inhibitors decreased the intracellular total antioxidant capacity of paclitaxel-treated cells. The reason for the decrease in total antioxidant capacity of paclitaxel-treated cells caused by these inhibitors is not clear at this moment. Our results showed that paclitaxel treatment increased O_2^- levels and SOD decreased production of H_2O_2 in paclitaxel-treated cells. The inhibition of SOD by 2-ME increased paclitaxel-induced apoptosis. These results suggest that paclitaxel may increase intracellular H_2O_2 levels by elevating O_2^- levels. Interference in microtubule dynamics is known to disrupt redox signaling. Cytoskeletal disruption can lead to activation of NADPH oxidase and the production of intracellular ROS (29). More research is needed to elucidate the mechanisms of how paclitaxel induces the generation of H_2O_2 , O_2^- , and NO.

H_2O_2 is known to produce the $\cdot OH$ radical in the presence of Fenton metals. O_2^- can also react rapidly with $NO\cdot$ to form peroxynitrite, a highly reactive species. There are many antioxidants that exist within the extracellular space, cell membrane, and cytosol. Cooperation among the different antioxidants provides greater protection against oxidant attacks than any one compound alone. Thus, measuring the overall antioxidant capacity seems to give more biologically relevant information than that obtained from measuring individual antioxidant content. To quantify total antioxidant capacity, the capacity of hydrogen-donating molecules that can reduce cation radicals generated by oxidation of 2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) was measured in cell extracts. This measurement presumably covers most radicals produced by O_2^- , H_2O_2 , and NO. Our results imply that tumor sensitivity to paclitaxel chemotherapy in a clinical setting might be predictable if total antioxidant

capacity measurements can be determined from tumor specimens, thus providing a way of tailoring cytotoxic therapy to each individual patient.

It has been reported that paclitaxel can induce DNA single-strand breaks (30–32). Our data indicate that paclitaxel may induce oxidative DNA damage by generating increased levels of H_2O_2 and NO. On DNA damage, cells may halt their progression in the cell cycle to repair damage. They may also initiate programmed cell death or allow the cell cycle to proceed without repairing damages even in the presence of profuse mutations or molecular alterations. The causal relationship between DNA damage and G_2 -M arrest, or between DNA damage and subsequent apoptosis in paclitaxel-treated cells, remains largely unknown. Here, we showed that in T24 cells, paclitaxel induced oxidative DNA adducts at a concentration as low as 0.02 μ mol/L. This level of paclitaxel is lower than the required concentration needed for inducing nuclear fragmentation, sub- G_1 accumulation, and G_2 -M arrest. Indeed, this finding may explain why gene mutation accumulation from minor DNA insults may facilitate the occurrence of drug-resistant cell clones rather than cell death.

Our results have a number of clinical implications. Because paclitaxel cytotoxicity can be significantly reduced by an antioxidant such as selenium, it is imperative to determine whether concurrent administration of antioxidants from over-the-counter food supplements may attenuate the efficacy of paclitaxel chemotherapy in clinical settings. It is feasible, however, to enhance the effects of paclitaxel treatment in clinical practice by applying agents that reduce intracellular antioxidant capacity, such as PD98059, U0126, LY294002, BSO, 2-ME, and As_2O_3 . More importantly, chemosensitivity to paclitaxel may be determined by taking total antioxidant capacity measurements from clinical tumor samples. This, in turn, may then improve treatment outcomes by selecting out potentially responsive patients.

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