Available online at www.sciencedirect.com
SCIENCE DIRECT®
European Journal of Pharmacology 489 (2004) 3–11
www.elsevier.com/locate/ejphar

Phorbol myristate induces apoptosis of taxol-resistant sarcoma cells in vitro

Zhi-ping Zong[a,*], Shinobu Matsui[a], Shogo Katsuda[b], Jian-feng Han[a], Kohzaburo Fujikawa-Yamamoto[a]

[a] Medical Research Institute, Division of Basic Science, Kanazawa Medical University, Uchinada-machi, Ishikawa 920-0293, Japan
[b] Department of Pathology, Kanazawa Medical University, Uchinada-machi, Ishikawa 920-0293, Japan

Received 13 November 2003; received in revised form 10 February 2004; accepted 20 February 2004

Abstract

Taxol was found to induce polyploidization and apoptosis in cultured methylcholanthrene-induced sarcoma cells (Meth-A cells), but some of the cells in G1 phase were not affected. We refer to these cells as taxol-resistant cells. Phorbol 12-myristate 13-acetate (PMA), a protein kinase C (PKC) regulator, was used to test the taxol-resistant cells. Many of the taxol-resistant cells disappeared after treatment with taxol in the presence of PMA. To explore the mechanism of this effect, we employed flow cytometry to determine levels of p53, p21, bcl-2 and caspase proteins in the taxol-resistant cells, and found that the expression of the bcl-2 protein was markedly decreased and the expression of the caspase protein markedly increased after treatment with taxol in the presence of PMA. These findings suggest that PMA enhances the sensitivity of taxol-resistant cells to taxol, and taxol treatment in the presence of PMA induces the apoptosis of taxol-resistant cells by inhibiting the expression of the bcl-2 protein and increasing the expression of the caspase protein.

Keywords: Taxol-resistant cell; Polyploidization; PMA; Apoptosis; bcl-2

1. Introduction

Taxane paclitaxel (taxol), identified by Wani et al. (1971), is a diterpenoid plant product isolated from the bark of Pacific yew trees (*Taxus brevifolia*) whose principal targets in cells are spindle microtubules responsible for the segregation of duplicated chromosomes to daughter cells at mitosis (Goncalves et al., 2001). Taxol binds reversibly to tubulin along the surface of microtubules where it increases the rate at which the tubulin monomer is polymerized and stabilizes the microtubule cytoskeleton against depolymerization (Arnal and Wade, 1995), resulting in the arrest of the cell cycle at metaphase/anaphase transition and cell death (Ling et al., 1998; Torres and Horwitz, 1998; Wang et al., 1998a). Taxol has achieved resounding success as a valuable chemotherapeutic agent used for the treatment of many types of cancer, including ovarian (Chuang et al., 2003; Parmar et al., 2003), breast (Chang et al., 2003), and non-small-cell lung tumors (O'Brien et al., 2003), as well as head and neck carcinomas (Posner and Lefebvre, 2003). However, as with other antitumor agents, drug resistance has been noted during and after chemotherapy with taxol (Yu et al., 1998; Kavallaris et al., 1999).

The resistance of tumor cells to chemotherapy is a major problem in the treatment of cancer. A search for the mechanisms by which tumors resist various treatments is being made worldwide. It is known that when confronted with an environment unfavorable for growth, eukaryotic cells stop dividing, leave the cell cycle, and enter a stable quiescent state often referred to as the G0 phase in which they remain viable for extended periods and from which they can re-enter the cell cycle (Wei et al., 1993). Entry into a quiescent state is believed to be a major reason why tumor cells resist some chemotherapeutic agents. Inducing tumor cells to re-enter the cell cycle from this quiescent state is a major goal in clinical practice.

Protein kinase C (PKC), a multigene family of enzymes consisting of at least ten different calcium and lipid-regulated protein kinases in addition to the PKC-related kinases,

* Corresponding author. Tel.: +81-76-286-2211x3970; fax: +81-76-286-3652.
E-mail address: zong3961@kanazawa-med.ac.jp (Z. Zong).

0014-2999/$ - see front matter © 2004 Elsevier B.V. All rights reserved.
doi:10.1016/j.ejphar.2004.02.027

is believed to play a crucial role in the signal transduction involved in the regulation of numerous cellular functions (Nishizuka, 1986, 1992). PKC-mediated phosphorylation is also believed to regulate membrane functions through translocation from the cytosol to cell membrane and nuclear membrane in response to activators (Sibley and Lefkowitz, 1985; Hartwig et al., 1992; Singh et al., 1994; Ikizawa et al., 1996). There have been lines of evidence demonstrating that cell-permeable diacylglycerol (DAG) derivatives, which activate PKC (Rosengurt et al., 1984), and phorbol esters, which mimic the activating effect of DAG, induce the transcription of a wide array of genes (Castagna et al., 1982; Colamonici et al., 1986).

The present study investigated whether phorbol ester induces taxol-resistant tumor cells to re-enter the cell cycle and/or undergo cell death. We employed flow cytometry to determine DNA content and found that some cells in G1/G0 phase with a 2C (C = haploid) DNA content persisted after taxol treatment. We refer to these cells as taxol-resistant cells. Following combined treatment with phorbol 12-myristate 13-acetate (PMA), the number of taxol-resistant cells decreased markedly.

2. Materials and methods

2.1. Reagents

Taxol and PMA were purchased from Sigma (St Louis, MO, USA) and dissolved in dimethyl sulfoxide. Taxol was stored in the dark at 4 °C and PMA was stored at −20 °C. Antibodies against 5-bromo-2′-deoxyuridine (BrdU), mitotic phosphoprotein monoclonal-2 (MPM2), p53, p21, bcl-2 and the caspase proteins used in this study were purchased from Wako, (Tokyo, Japan). All other chemicals were of reagent grade and obtained from Sigma.

2.2. Cell line and culture conditions

Meth-A cells (a methylcholanthrene-induced sarcoma cell line, syngeneic to BALB/c mice) were grown in RPMI 1640 medium supplemented with 10% heat-inactivated fetal calf serum, 50 U/ml of penicillin and 50 μg/ml of streptomycin. The cells were maintained at 37 °C in a humidified atmosphere containing 5% CO_2 and 95% air. The viability of the cells used in these experiments was consistently more than 95% when evaluated by the Trypan blue exclusion method.

2.3. Experimental animals

Pathogen-free male BALB/c mice, 7 weeks old, were purchased from Sankyo Laboratory (Shizuoka, Japan). The mice were housed in animal quarters with controlled temperature (22–26 °C), humidity (50–60%), and lighting (12-h cycle). Five animals were used per experimental condition. All experiments were repeated at least twice and representative data are displayed.

2.4. Flow cytometric measurements

Flow cytometry was employed to determine the distribution of DNA content in Meth-A cells. After fixation in 70% ethanol, the cells were treated exhaustively with pancreatic RNase A and stained with 10 μg/ml of propidium iodide (PI) in phosphate-buffered saline (PBS). Fluorescence from individual cells was measured with a flow cytometer (Cytofluorograf system 50H, Ortho Instruments, NJ, USA) and a FACSort (Becton Dickinson Immunocytometry System, NJ, USA). The relative intensities of red fluorescence were measured and DNA histograms were obtained. The results were analyzed using CELLQuest software.

Flow cytometry was also employed to determine the expression of MPM2, p53, p21, bcl-2 and caspase proteins, and incorporation of BrdU by indirect immunofluorescence using the specific antibodies and the standard methodology (Zong et al., 1999). Meth-A cells were collected, washed in PBS, fixed with 1% paraformaldehyde for 30 min at room temperature and treated with 0.3% Triton X-100 for 10 min at 37 °C. The cells were then incubated with the primary antibodies for 24 h at 4 °C, washed in PBS containing 1% bovine serum albumin, and stained with secondary immunoglobulin G fluorescein isothiocyanate-conjugated antibodies. They were then washed again, resuspended in PBS containing RNase A and PI, and analyzed by flow cytometry.

2.5. In vivo experiments

Meth-A cells (1×10^5) were injected i.p. into the BALB/c mice. An i.p. injection of PMA (for the PMA-treated group), taxol (taxol-treated group), PMA plus taxol (PMA plus taxol-treated group), or an equal volume of solvent as a control (control group) was administered to the animals at 0.05 μg/mouse (PMA) and/or 0.5 mg/mouse (taxol), every other day, four times after 3 days of inoculation with Meth-A cells. Mice were checked daily for survival. In two different experiments, ascites fluid was collected at indicated times and its volume evaluated.

3. Results

3.1. Taxol induces polyploidization and apoptosis in Meth-A cells

The time course and dose response of the effect of taxol on DNA content were examined in exponentially growing Meth-A cells. The cells were stained with PI and subjected to flow cytometry. Fig. 1 shows the histograms. The first

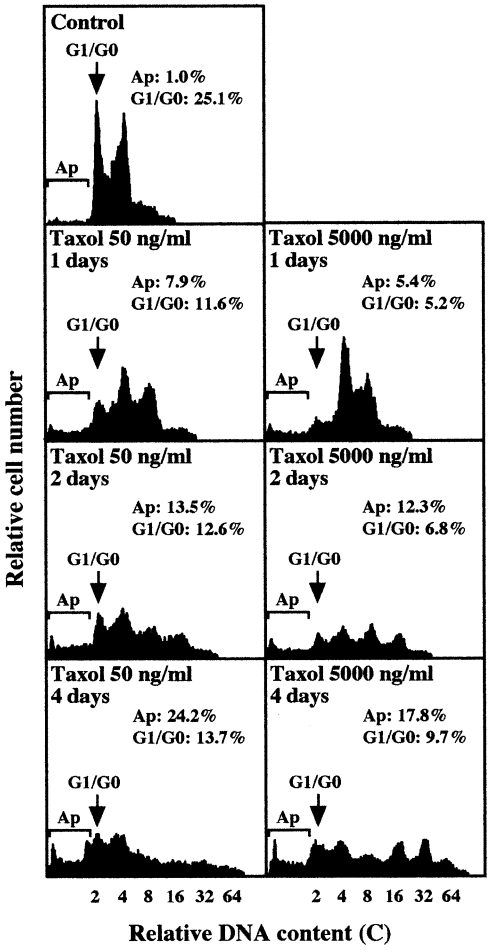

Fig. 1. Taxol induces polyploidization and apoptosis in Meth-A cells. Meth-A cells were treated with 0, 50 or 5000 ng/ml of taxol for 4 days. The cells were collected and the distribution of DNA content was determined by flow cytometry. C: haploid DNA content. Ap: apoptosis.

peak was produced by cells with a 2C, the second with a 4C, the third with an 8C, the fourth with a 16C, the fifth with a 32C and the sixth with a 64C DNA content, respectively. The cells with 2C and 4C DNA contents are referred to as the first cell cycle cells, and those with 4C and 8C as the second, 8C and 16C as the third, 16C and 32C as the fourth, and 32C and 64C as the fifth cell cycle cells. The 4C, 8C, 16C and 32C peaks were produced by cells in the G2 phase and G1 phase of the next cell cycle, respectively. The hypodiploid population was composed of apoptotic bodies (Del Bino et al., 1992). With continued incubation after exposure to 50 or 5000 ng/ml of taxol for 4 days, the DNA content progressively increased and the main peaks shifted from 2C in the control cells to 4C in the 50 ng/ml taxol-treated cells and to 32C in the 5000 ng/ml taxol-treated cells at the 4-day time point.

For the 1-day treatment, cells in the first peak produced by the diploidy cell population in G1/G0 decreased from 25.1% in the control cells to 11.6% in the 50 ng/ml and to 5.2% in the 5000 ng/ml taxol-treated cells. The extension of taxol treatment from 1 to 4 days did not markedly change the percentage in the 50 ng/ml (11.6% to 13.7%) and slightly increased the percentage in the 5000 ng/ml taxol-treated cells (5.2% to 9.7%). The per-

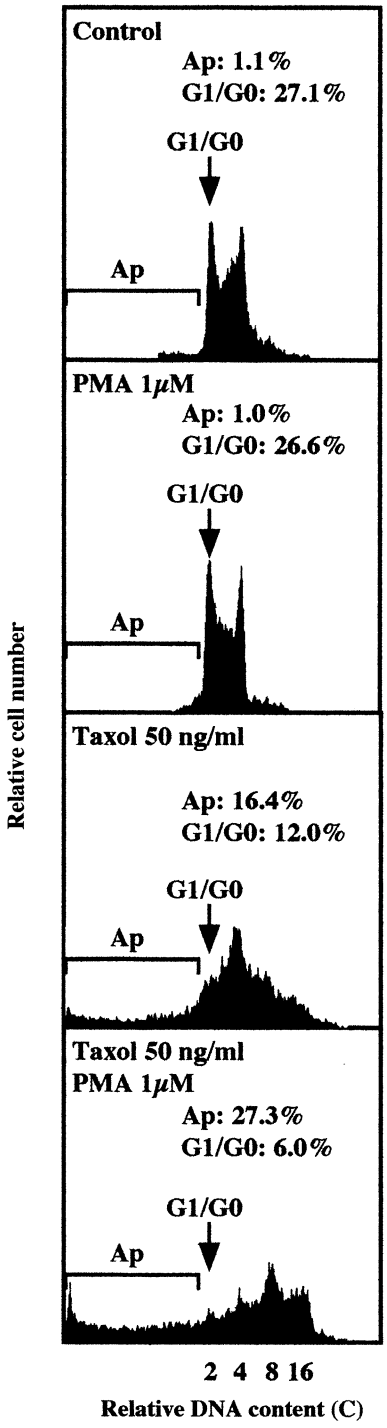

Fig. 2. PMA potentiates taxol-induced polyploidization and apoptosis in Meth-A cells. Meth-A cells were treated with 0, 1 μM of PMA, 50 ng/ml of taxol, or 50 ng/ml of taxol in the presence of 1 μM of PMA for 60 h. The cells were collected and the distribution of DNA content was determined by flow cytometry. C: haploid DNA content. Ap: apoptosis.

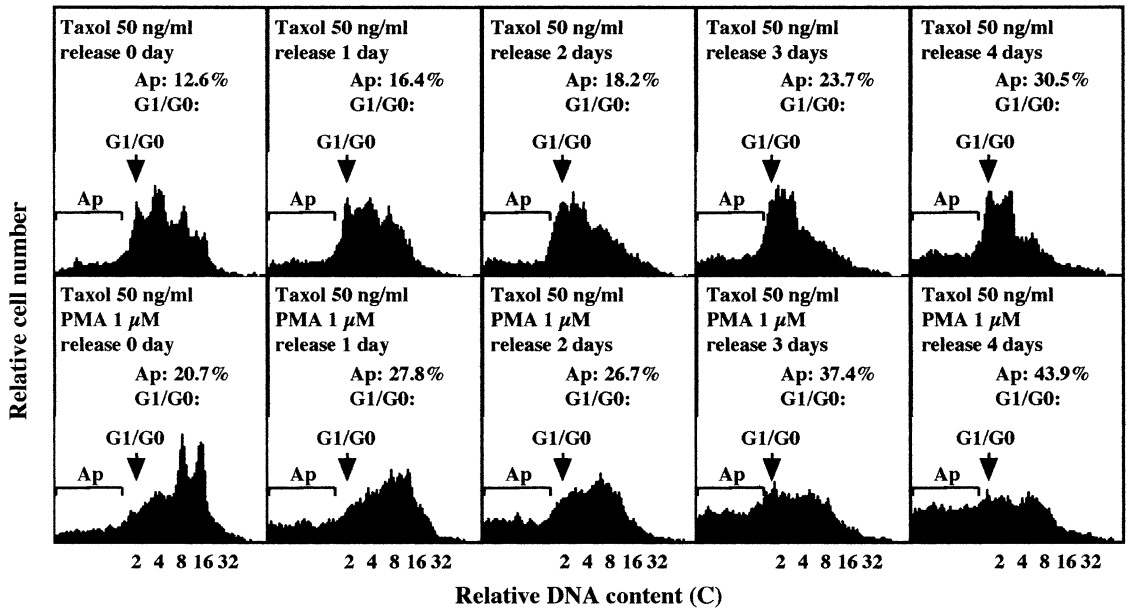

Fig. 3. Changes in PMA and taxol-treated Meth-A cells when released from the drugs. Meth-A cells were released from the drugs into normal growth medium after treatment with taxol or taxol plus PMA for 60 h. The cells were collected and the distribution of DNA content was determined by flow cytometry. C: haploid DNA content. Ap: apoptosis.

centage of the hypodiploid population was markedly increased in a time-dependent manner in both taxol-treated groups.

These results suggest that, under the taxol-treated conditions, taxol-sensitive cells are arrested in metaphase, and by some unknown mechanisms escape the arrest, bypass cellular division, enter into a new cycle of DNA replication, and become polyploid cells, which then die in an apoptotic manner. Taxol-resistant cells leave the cell cycle and enter G0 phase. In the present study, the cells that persisted in the

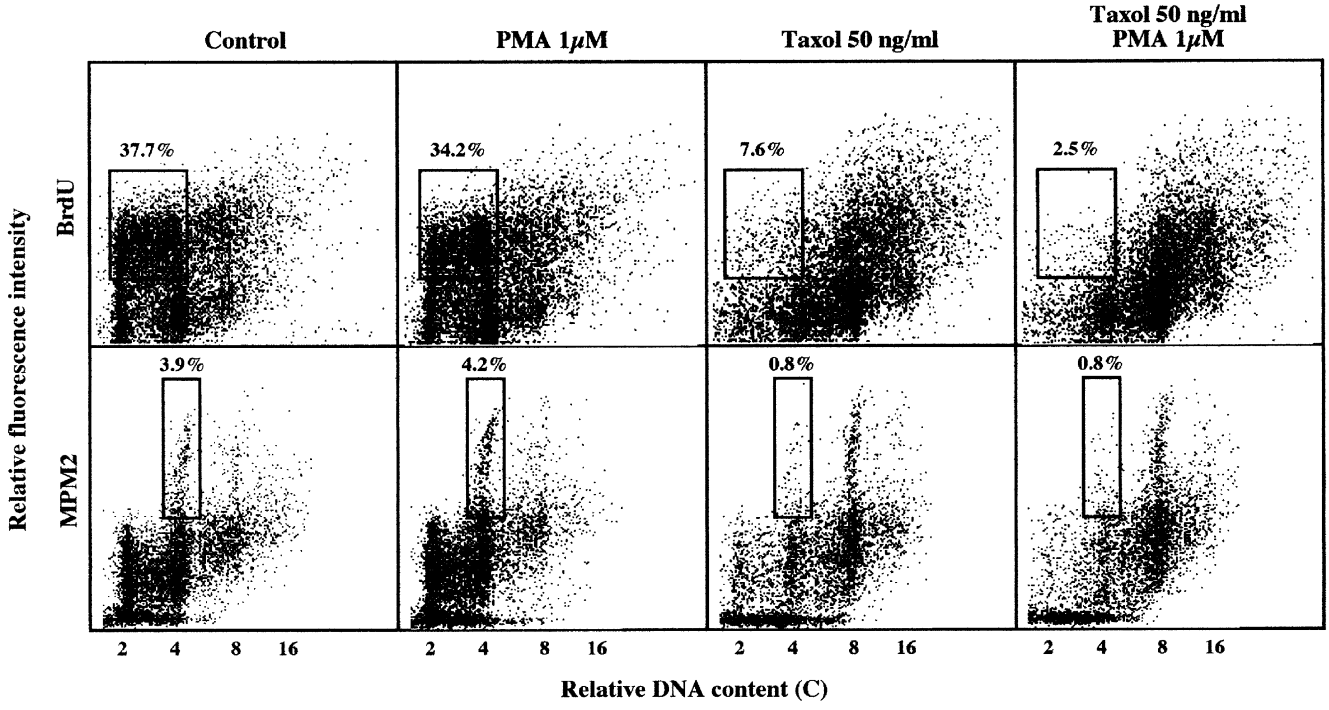

Fig. 4. Effects of taxol in the presence of PMA on S and M phases. Meth-A cells were treated with 0, 1 μM of PMA, 50 ng/ml of taxol, or 50 ng/ml of taxol in the presence of 1 μM of PMA for 60 h. The cells were collected, and the incorporation of BrdU and the expression of MPM2 protein were determined by flow cytometry. C: haploid DNA content.

first peak produced by the diploidy cell population in G1/G0 after the treatment with taxol are referred to as taxol-resistant cells.

3.2. PMA potentiates taxol-induced polyploidization and apoptosis in Meth-A cells

Effects of PMA on the taxol-induced polyploidization and apoptosis, and the taxol-resistant cells were examined (Fig. 2). Treatment with 1 μM PMA for 60 h did not markedly interfere with the progression of the cell cycle, but in combination with 50 ng/ml of taxol, PMA potentiated polyploidization. The main peak shifted from 4C in the 50 ng/ml taxol-treated cells to 8C in the 1 μM PMA plus 50 ng/ml taxol-treated cells. At the same time, the number of taxol-resistant cells markedly decreased (from 12% in the 50 ng/ml taxol-treated cells to 6% in the 1 μM PMA plus 50 ng/ml taxol-treated cells) and the hypodiploid population markedly increased (from 16.4% in the 50 ng/ml taxol-treated cells to 27.3% in the 1 μM PMA plus 50 ng/ml taxol-treated cells).

When the cells were released from the drug treatment into normal growth medium after exposure to taxol or taxol plus PMA for 60 h, histograms obtained from the taxol-treated cells gradually resembled those obtained from the control cells (Figs. 1 and 3). Namely, at the 4-day time point, the cells with the 2C and 4C DNA contents produced two main peaks, the cells with an 8C DNA content markedly decreased and produced a small peak, and the cells with a 16C DNA content almost disappeared. In contrast, the histograms obtained from the PMA plus taxol-treated cells showed a different pattern. Namely, until to the 4-day point, the number of cells with 2C DNA content merely increased to a lesser extent, whereas the cells with 8C and 16C DNA contents markedly decreased and the hypodiploid population markedly increased.

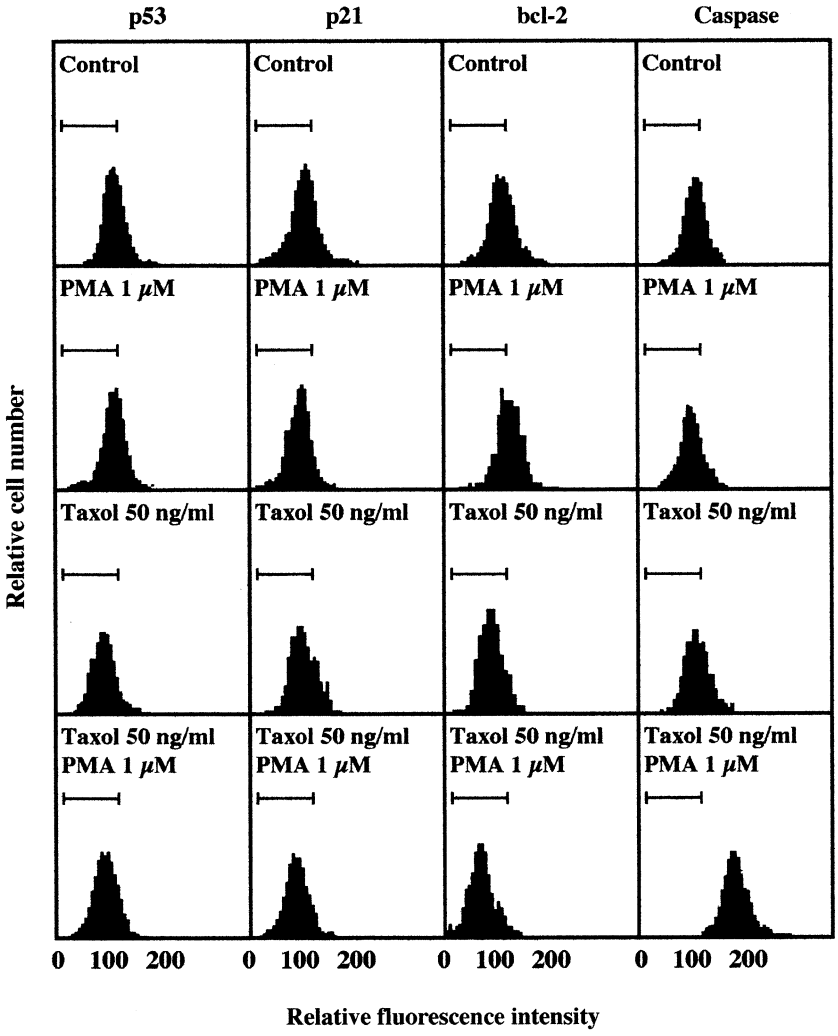

Fig. 5. Analysis of expression of p53, p21, bcl-2 and caspase proteins by flow cytometry. The cells were treated 0, 1 μM of PMA, 50 ng/ml of taxol, or 50 ng/ml of taxol in the presence of 1 μM of PMA for 60 h, simultaneously stained with PI and monoclonal antibodies against p53, p21, bcl-2 or caspase protein, and subjected to flow cytometry. The cells with 2C were gated, and the expression of the proteins displayed.

These results indicate that PMA potentiates the taxol-induced polyploidization and apoptosis, and suggest that PKC plays an important role in up-regulating the sensitivity of Meth-A cells including taxol-resistant cells to taxol.

3.3. Effects of taxol in the presence of PMA on S and M phases

To clarify the S and M phases, Meth-A cells were simultaneously stained with PI and monoclonal antibodies against BrdU (as a hallmark of S phase) or MPM2 (as a hallmark of M phase) protein, and flow cytometry was used to quantify the incorporation of BrdU and the expression of MPM2 protein (Fig. 4). Treatment with 1 μM PMA for 60 h did not markedly interfere with the incorporation of BrdU. The incorporation of BrdU mainly occurred in the third cell cycle with some incorporation in the first (7.6%) following treatment with 50 ng/ml of taxol. Addition of PMA potentiated the effect of taxol in reducing the incorporation that occurred in the first cell cycle (2.5%). Different from the incorporation of BrdU, treatment with 1 μM PMA for 60 h not only did not markedly interfere with the expression of MPM2 protein in the control cells, but also did not potentiate the expression of MPM2 protein in the taxol-treated cells.

3.4. Expression of p53, p21, bcl-2 and caspase proteins in taxol-resistant cells

To investigate the mechanism whereby taxol-resistant cells disappear after taxol treatment in the presence of PMA, we examined the expression of p53, p21, bcl-2 and caspase proteins in cells with a 2C DNA content. Flow cytometry was used to determine quantitative changes. Meth-A cells were simultaneously stained with PI and monoclonal antibody against p53, p21, bcl-2 or caspase protein, and subjected to flow cytometry. The cells with 2C DNA content were gated, and the expression of p53, p21, bcl-2 or caspase protein was determined. As shown in Fig. 5, p53 protein was present in cells with a 2C DNA content, but the quantitative change was not remarkable in these cells treated with 1 μM PMA for 60 h. At 50 ng/ml, taxol slightly decreased the expression. Addition of 1 μM PMA did not influence the effect of taxol. Effects of PMA and taxol on the expression of p21 protein were similar to those on the expression of p53 protein. These results suggest that p53-related cell death was not involved in the disappearance of the taxol-resistant cells.

When we examined the expression of bcl-2 protein, we found that the quantitative change in bcl-2 protein was slightly potentiated in the cells treated with PMA. In contrast, taxol slightly down-regulated the expression, whereas PMA enhanced the effect of taxol. Caspase was not quantitatively affected in the cells treated with taxol. However, its expression was slightly decreased in the cells treated with PMA and markedly increased in the cells treated with taxol in the presence of PMA. These results support the possibility that a decrease of bcl-2 protein expression and increase of caspase protein expression are responsible for the disappearance of the taxol-resistant cells.

3.5. Survival of mice bearing Meth-A cells

Generally speaking, survival is comparable with tumorigenicity (or mitotic capacity). To verify this, we examined the survival of mice bearing Meth-A cells. Fig. 6A shows survival rates. In control and PMA-treated groups, mice started to die from day 14 after an i.p. injection of Meth-A cells and by day 15 were all dead. In contrast, those in the taxol-treated group died from 30 to 39 days, significantly later than the mice in the control and PMA-treated groups. In the PMA plus taxol-treated group, mice died from 30 to 72 days, and moreover, 20% were still alive at day 81. Furthermore, at day 81, the living mice were sacrificed and dissected, with no abnormal findings.

In two different experiments, the mice were sacrificed under ether anesthesia, and ascites fluid was collected and its volume measured at day 12 before mice begin to die in any of the four groups (Fig. 6B), and at day 25 after mice had died in the control and PMA-treated groups but before they had died in the taxol- and PMA plus taxol-treated

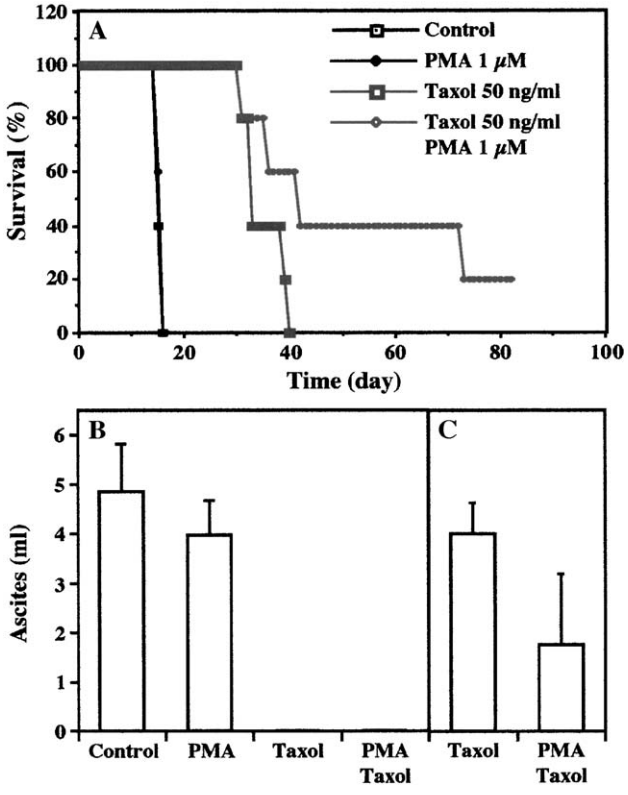

Fig. 6. Survival of mice bearing Meth-A cells. An i.p. injection of PMA or taxol was administered to the BALB/c mice after 3 days of inoculation with Meth-A cells inoculation, as described in Materials and methods. Mice were checked daily for survival, and the ascites fluid was collected and its volume measured.

groups (Fig. 6C). At day 12, the volume of ascites was smaller in the PMA-treated group than control group, and no ascites was observed in the taxol- and PMA plus taxol-treated groups. However at day 25, the volume of ascites was increased, and larger in the taxol-treated group than the PMA plus taxol-treated group.

4. Discussion

Resistance to antineoplastic drugs is a major problem in the treatment of cancer. In the same way, multidrug resistance imposes substantial limits on tumor responsiveness to taxol in a clinical setting. Experiments in vitro have yielded a number of taxol-resistant sublines used to examine the mechanisms of resistance to taxol (Cabral et al., 1981; Roy and Horwitz, 1985; Bhalla et al., 1994; Duan et al., 1999; Goncalves et al., 2001; Zhou et al., 2001; Martello et al., 2003). In the present study, 50 ng/ml of taxol induced polyploidization of Meth-A cells, but some of the cells with 2C DNA content persisted throughout the 4-day treatment. However in the presence of PMA, the number of cells with a 2C DNA content referred to here as taxol-resistant cells was reduced by half. Moreover, when the cells were released from treatment into normal growth medium, the taxol-resistant cells and the cells with a 4C DNA content gradually increased and produced two main peaks similar to the control group, whereas in the PMA plus taxol-treated cells, the number of taxol-resistant cells merely increased to a lesser extent and the hypodiploid population markedly increased. In in vivo experiments, mice bearing Meth-A cells survived longer when treated with PMA plus taxol than with taxol only. These findings demonstrated that the PMA–taxol combination effectively overcomes taxol resistance in these cells, and suggest that PKC plays an important role in up-regulating the sensitivity of Meth-A cells including the taxol-resistant cells to taxol.

PKC has been reported to be closely associated with the pharmacological effects of taxol. Le et al. (2003) reported that taxol induced the phosphorylation and inactivation of 70-kDa ribosomal S6 kinase (p70S6K), and that the phosphorylation requires both de novo RNA and protein synthesis via multiple signaling pathways involving PKC. Wang et al. (1998b) reported that the PKC activator bryostatin 1 increased the susceptibility of U937 cells to taxol-induced apoptosis and inhibition of clonogenicity. PKC has also been reported to be closely associated with multidrug resistance. Yoshida et al. (2003) reported that K562 leukemia cells exhibiting an increase in PKC alpha expression became sensitive to gnidimacrin. Narvaez et al. (2003) reported that the induction of apoptosis by 1,25-dihydroxyvitamin D3 in a MCF-7 Vitamin D3-resistant variant (a human breast cancer cell line) could be sensitized by PMA. These results support those obtained in the present study.

The p53 protein is the product of a 20-kb gene located on the short arm of chromosome 17 and a 393-amino-acid nuclear phosphoprotein. p53's multifaceted activities in the G1, M and S phases of the cell cycle involving p21, one of its important effectors, and effect on apoptosis have been reported (Chang et al., 1993; Waga et al., 1994; Rellamy et al., 1997). To explore the possible mechanism by which taxol treatment in the presence of PMA abolished the taxol-resistant cells, we investigated the expression of p53 and p21 proteins in the taxol-resistant cells using flow cytometry. We found that the expression of these two proteins was not affected by treatment with PMA, but decreased by treatment with taxol or taxol in the presence of PMA. These results suggest that p53-related cell death was not involved in the disappearance of the taxol-resistant cells. Reversely, the decrease in expression of p53 and p21 proteins accelerated the progression of the cell cycle and potentiated the polyploidization.

Evidence suggesting the involvement of a variety of biologically active substances other than p53 protein in taxol resistance includes: (1) overexpression of interleukin-2 receptor alpha (Kuhn et al., 2003); (2) overexpression of interleukin-6 (Yusuf et al., 2003); (3) heat shock protein 90 (Solit et al., 2003); (4) overexpression of the multidrug resistance (MDR-1) gene (Yusuf et al., 2003); (5) molecular changes in the target molecule (beta-tubulin) (Yusuf et al., 2003); (6) changes in apoptotic regulatory and mitosis checkpoint proteins (Yusuf et al., 2003); and (7) bcl-2 family proteins. The bcl-2 protein composed of 239 amino acids is expressed in mitochondria (Hockenbery et al., 1990), the endoplasmic reticulum and the nuclear membrane (Jacobson et al., 1993), and has been shown to prevent the activation of downstream caspase cascade and to prolong cell survival by inhibiting apoptosis (Fujimura et al., 2003; Gabriel et al., 2003; Haupt et al., 2003; Yamashita et al., 2003). Therefore, we next investigated the expression of bcl-2 and caspase proteins in the taxol-resistant cells using flow cytometry. We found that taxol treatment slightly, and taxol treatment in the presence of PMA markedly, inhibited the expression of bcl-2 protein. Moreover, taxol treatment in the presence of PMA markedly increased the expression of caspase protein. It is important to assess whether some of the genes, which encode proteins whose expression, were investigated in this study could harbor mutations. The mutations in the p53, p21, bcl-2 and caspase genes will be investigated in our laboratory.

Taken together, the results of the present study show that, in this cell line, taxol treatment in the presence of PMA induces the disappearance of taxol-resistant cells in several ways, i.e., PMA up-regulates the sensitivity of the taxol-resistant cells to taxol, and taxol treatment in the presence of PMA induces apoptosis of the taxol-resistant cells by inhibiting the expression of bcl-2 protein and increasing the expression of caspase protein. In the previous report, we have shown that PMA potentiates taxol-induced polyploidization in at least two ways, i.e., accelerating the progression of the cell cycle by up-regulation

of G1 and G2 checkpoint-related proteins and potentiating spindle disorganization, which blocks mitosis (Zong et al., 2000). These findings suggest that appropriate drug combinations with taxol may be clinically useful. We expect the possible application of these results in clinical trials for cancer therapy.

References

Arnal, I., Wade, R.H., 1995. How does taxol stabilize microtubules? Curr. Biol. 5, 900–908.

Bhalla, K., Huang, Y., Tang, C., Self, S., Ray, S., Mahoney, M.E., Ponnathpur, V., Tourkina, E., Ibrado, A.M., Bullock, G., 1994. Characterization of a human myeloid leukemia cell line highly resistant to taxol. Leukemia 8, 465–475.

Cabral, F., Abraham, I., Gottesman, M.M., 1981. Isolation of a taxol-resistant Chinese hamster ovary cell mutant that has an alteration in alpha-tubulin. Proc. Natl. Acad. Sci. U. S. A. 78, 4388–4391.

Castagna, M., Takai, Y., Kaibuchi, K., Sano, K., Kikkawa, U., Nishizuka, Y., 1982. Direct activation of calcium-activated, phospholipid-dependent protein kinase by tumor-promoting phorbol esters. J. Biol. Chem. 257, 7847–7851.

Chang, F., Syrjanen, S., Tervahauta, A., Syrjanen, K., 1993. Tumourigenesis associated with the p53 tumour suppressor gene. Br. J. Cancer 68, 653–661.

Chang, J.C., Wooten, E.C., Tsimelzon, A., Hilsenbeck, S.G., Gutierrez, M.C., Elledge, R., Mohsin, S., Osborne, C.K., Chamness, G.C., Allred, D.C., O'Connell, P., 2003. Gene expression profiling for the prediction of therapeutic response to docetaxel in patients with breast cancer. Lancet 362, 362–369.

Chuang, T.C., Lee, Y.J., Liu, J.Y., Lin, Y.S., Li, J.W., Wang, V., Law, S.L., Kao, M.C., 2003. EBNA1 may prolong G(2)/M phase and sensitize HER2/neu-overexpressing ovarian cancer cells to both topoisomerase II-targeting and paclitaxel drugs. Biochem. Biophys. Res. Commun. 307, 653–659.

Colamonici, O.R., Trepe, J.B., Vidal, C.A., Neckers, L.M., 1986. Phorbol ester induces c-sis gene transcription in stem cell line K-562. Mol. Cell. Biol. 6, 1847–1850.

Del Bino, G., Bruno, S., Yi, P.N., Darzynkiewicz, Z., 1992. Apoptotic cell death triggered by camptothecin or teniposide: the cell cycle specificity and effects of ionizing radiation. Cell Prolif. 25, 537–548.

Duan, Z., Feller, A.J., Toh, H.C., Makastorsis, T., Seiden, M.V., 1999. TRAG-3, a novel gene, isolated from a taxol-resistant ovarian carcinoma cell line. Gene 229, 75–81.

Fujimura, S., Suzumiya, J., Yamada, Y., Kuroki, M., Ono, J., 2003. Down-regulation of Bcl-x(L) and activation of caspases during retinoic acid-induced apoptosis in an adult T-cell leukemia cell line. Hematol. J. 4, 328–335.

Gabriel, B., Sureau, F., Casselyn, M., Teissie, J., Petit, P.X., 2003. Retroactive pathway involving mitochondria in electroloaded cytochrome *c*-induced apoptosis. Protective properties of Bcl-2 and Bcl-(XL). Exp. Cell Res. 289, 195–210.

Goncalves, A., Braguer, D., Kamath, K., Martello, L., Briand, C., Horwitz, S., Wilson, L., Jordan, M.A., 2001. Resistance to Taxol in lung cancer cells associated with increased microtubule dynamics. Proc. Natl. Acad. Sci. U. S. A. 98, 11737–11742.

Hartwig, J.H., Thelen, M., Rosen, A., Janmey, P.A., Nairn, A.C., Aderem, A., 1992. MARCKS is an actin filament crosslinking protein regulated by protein kinase C and calcium-calmodulin. Nature 356, 618–622.

Haupt, S., Berger, M., Goldberg, Z., Haupt, Y., 2003. Apoptosis - the p53 network. J. Cell. Sci. 116, 4077–4085.

Hockenbery, D., Nunez, G., Milliman, C., Schreiber, R.D., Korsmeyer, S.J., 1990. Bcl-2 is an inner mitochondrial membrane protein that blocks programmed cell death. Nature 348, 334–336.

Ikizawa, K., Kajiwara, K., Basaki, Y., Koshio, T., Yanagihara, Y., 1996. Evidence for a role of phosphatidylinositol 3-kinase in IL-4-induced germline C epsilon transcription. Cell. Immunol. 170, 134–140.

Jacobson, M.D., Burne, J.F., King, M.P., Miyashita, T., Reed, J.C., Raff, M.C., 1993. Bcl-2 blocks apoptosis in cells lacking mitochondrial DNA. Nature 361, 365–369.

Kavallaris, M., Burkhart, C.A., Horwitz, S.B., 1999. Antisense oligonucleotides to class III beta-tubulin sensitize drug-resistant cells to Taxol. Br. J. Cancer 80, 1020–1025.

Kuhn, D.J., Smith, D.M., Pross, S., Whiteside, T.L., Dou, Q.P., 2003. Overexpression of interleukin-2 receptor alpha in a human squamous cell carcinoma of the head and neck cell line is associated with increased proliferation, drug resistance, and transforming ability. J. Cell. Biochem. 9, 824–836.

Le, X.F., Hittelman, W.N., Liu, J., Mc Watters, A., Li, C., Mills, G.B., Bast Jr., R.C., 2003. Paclitaxel induces inactivation of p70 S6 kinase and phosphorylation of Thr421 and Ser424 via multiple signaling pathways in mitosis. Oncogene 22, 484–497.

Ling, Y.H., Consoli, U., Tornos, C., Andreeff, M., Perez-Soler, R., 1998. Accumulation of cyclin B1, activation of cyclin B1-dependent kinase and induction of programmed cell death in human epidermoid carcinoma KB cells treated with taxol. Int. J. Cancer 75, 925–932.

Martello, L.A., Verdier-Pinard, P., Shen, H.J., He, L., Torres, K., Orr, G.A., Horwitz, S.B., 2003. Elevated levels of microtubule destabilizing factors in a Taxol-resistant/dependent A549 cell line with an alpha-tubulin mutation. Cancer Res. 63, 1207–1213.

Narvaez, C.J., Byrne, B.M., Romu, S., Valrance, M., Welsh, J., 2003. Induction of apoptosis by 1,25-dihydroxyvitamin D3 in MCF-7 vitamin D3-resistant variant can be sensitized by TPA. J. Steroid Biochem. Mol. Biol. 84, 199–209.

Nishizuka, Y., 1986. Studies and perspectives of protein kinase C. Science 233, 305–312.

Nishizuka, Y., 1992. Intracellular signaling by hydrolysis of phospholipids and activation of protein kinase C. Science 258, 607–614.

O'Brien, M.E., Splinter, T., Smit, E.F., Biesma, B., Krzakowski, M., Tjan-Heijnen, V.C., Van Bochove, A., Stigt, J., Smid-Geirnaerdt, M.J., Debruyne, C., Legrand, C., Giaccone, G., 2003. Carboplatin and paclitaxel (Taxol) as an induction regimen for patients with biopsy-proven stage IIIA N2 non-small cell lung cancer. an EORTC phase II study. Eur. J. Cancer 39, 1416–1422.

Parmar, M.K., Ledermann, J.A., Colombo, N., du Bois, A., Delaloye, J.F., Kristensen, G.B., Wheeler, S., Swart, A.M., Qian, W., Torri, V., Floriani, I., Jayson, G., Lamont, A., Trope, C., 2003. Paclitaxel plus platinum-based chemotherapy versus conventional platinum-based chemotherapy in women with relapsed ovarian cancer: the ICON4/AGO-OVAR-2.2 trial. Lancet 361, 2099–2106.

Posner, M.R., Lefebvre, J.L., 2003. Docetaxel induction therapy in locally advanced squamous cell carcinoma of the head and neck. Br. J. Cancer 88, 11–17.

Rellamy, C.O.C., Clarke, A.R., Wyllie, A.H., 1997. p53 deficiency in liver reduces local control of survival and proliferation, but dose not affect apoptosis after DNA damage. FASEB J. 11, 591–599.

Rosengurt, E., Rodriquez-Pena, A., Coombs, M., Sinnet-Smith, J., 1984. Diacylglycerol stimulates DNA synthesis and cell division in mouse 3T3 cells: role of Ca2+-sensitive phospholipid-dependent protein kinase. Proc. Natl. Acad. Sci. U. S. A. 81, 5748–5752.

Roy, S.N., Horwitz, S.B., 1985. A phosphoglycoprotein associated with taxol resistance in J774.2 cells. Cancer Res. 45, 3856–3863.

Sibley, D.R., Lefkowitz, R.J., 1985. Molecular mechanisms of receptor desensitization using the beta-adrenergic receptor-coupled adenylate cyclase system as a model. Nature 317, 124–129.

Singh, K.R., Taylor, L.K., Campbell, X.Z., Fields, A.P., Neet, K.E., 1994. A bryostatin-sensitive protein kinase C required for nerve growth factor activity. Biochemistry 33, 542–551.

Solit, D.B., Basso, A.D., Olshen, A.B., Scher, H.I., Rosen, N., 2003. Inhibition of heat shock protein 90 function down-regulates Akt kinase and sensitizes tumors to Taxol. Cancer Res. 63, 2139–2144.

Torres, K., Horwitz, S.B., 1998. Mechanisms of Taxol-induced cell death are concentration dependent. Cancer Res. 58, 3620–3626.

Waga, S., Hannon, G.J., Beach, D., Stillman, B., 1994. The p21 inhibitor of cyclin-dependent kinases controls DNA replication by interaction with PCNA. Nature 369, 574–578.

Wang, T.H., Wang, H.S., Ichijo, H., Giannakakou, P., Foster, J.S., Fojo, T., Wimalasena, J., 1998a. Microtubule-interfering agents activate c-Jun N-terminal kinase/stress-activated protein kinase through both Ras and apoptosis signal-regulating kinase pathways. J. Biol. Chem. 273, 4928–4936.

Wang, S., Guo, C.Y., Castillo, A., Dent, P., Grant, S., 1998b. Effect of bryostatin 1 on taxol-induced apoptosis and cytotoxicity in human leukemia cells (U937). Biochem. Pharmacol. 56, 635–644.

Wani, M.C., Taylor, H.L., Wall, M.E., Coggon, P., McPhail, A.T., 1971. Plant antitumor agents: VI. The isolation and structure of taxol, a novel antileukemic and antitumor agent from *Taxus brevifolia*. J. Am. Chem. Soc. 93, 2325–2327.

Wei, W., Nurse, P., Broek, D., 1993. Yeast cells can enter a quiescent state through G1, S, G2, or M phase of the cell cycle. Cancer Res. 53, 1867–1870.

Yamashita, S., Mita, S., Kato, S., Okado, H., Ohama, E., Uchino, M., 2003. Bcl-2 expression using retrograde transport of adenoviral vectors inhibits cytochrome c-release and caspase-1 activation in motor neurons of mutant superoxide dismutase 1 (G93A) transgenic mice. Neurosci. Lett. 350, 17–20.

Yoshida, M., Heike, Y., Ohno, S., Ikekawa, T., Wakasugi, H., 2003. Involvement of PKC betaII in anti-proliferating action of a new antitumor compound gnidimacrin. Int. J. Cancer 105, 601–606.

Yu, D., Liu, B., Jing, T., Sun, D., Price, J.E., Singletary, S.E., Ibrahim, N., Hortobagyi, G.N., Hung, M.C., 1998. Overexpression of both p185c-erbB2 and p170mdr-1 renders breast cancer cells highly resistant to taxol. Oncogene 16, 2087–2094.

Yusuf, R.Z., Duan, Z., Lamendola, D.E., Penson, R.T., Seiden, M.V., 2003. Paclitaxel resistance: molecular mechanisms and pharmacologic manipulation. Curr. Cancer Drug Targets 3, 1–19.

Zhou, J., Cheng, S.C., Luo, D., Xie, Y., 2001. Study of multi-drug resistant mechanisms in a taxol-resistant hepatocellular carcinoma QGY-TR 50 cell line. Biochem. Biophys. Res. Commun. 280, 1237–1242.

Zong, Z.P., Fujikawa-Yamamoto, K., Li, A.L., Yamaguchi, N., Chang, Y.G., Murakami, M., Odashima, S., Ishikawa, Y., 1999. Both low and high concentrations of staurosporine induce G1 arrest through down-regulation of cyclin E and cdk2 expression. Cell Struct. Funct. 24, 457–463.

Zong, Z.P., Fujikawa-Yamamoto, K., Li, A.L., Yamaguchi, N., Chang, Y.G., Murakami, M., Ishikawa, Y., 2000. Involvement of protein kinase C in taxol-induced polyploidization in a cultured sarcoma cell line. Eur. J. Pharmacol. 394, 181–187.