Paclitaxel-Induced Apoptosis in Human Gastric Carcinoma Cell Lines

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BACKGROUND. Gastric cancer is one of the most common cancers in Asia. Chemotherapy and radiation therapy have had limited success. Recently, paclitaxel has been found to be effective against a variety of cancers, including lung, breast, ovary, melanoma, and prostate. Whether paclitaxel is effective in the treatment of gastric cancer is not known and is worthy of investigation.

METHODS. Human gastric carcinoma cell lines NUGC-3 and SC-M1 were examined for response to paclitaxel treatment. Cancer cells were treated with paclitaxel (0.001, 0.01, 0.1, and 1 μM) for 1–3 days. Cell number was counted by hemocytometer and cell viability was determined by the trypan blue exclusion method. Cell cycle progression and expression of proliferating cell nuclear antigen (PCNA) were examined by flow cytometry. The percentage of apoptotic cells was determined after staining with hematoxylin and eosin.

RESULTS. Paclitaxel was cytotoxic to the two human gastric carcinoma cell lines examined. The growth-inhibiting dose was 0.01 μM. Paclitaxel-treated gastric carcinoma cells were arrested mainly in G_2/M phases before apoptosis. However, treatment with 0.01 μM of paclitaxel resulted in a decrease of cells at G_0/G_1 phases without an increase of cells at G_2/M phase indicating that paclitaxel was also cytotoxic to gastric carcinoma cells at G_0/G_1 phases. In addition, the expression of PCNA was significantly increased in 0.1 and 1 μM paclitaxel-treated cells, suggesting that DNA repair was increased in these cells.

CONCLUSIONS. Paclitaxel is effective in growth inhibition of gastric carcinoma cell lines in clinically attainable concentrations. Our results suggest that paclitaxel is a potential chemotherapeutic drug for gastric carcinoma. ***Cancer* 1996;77:14–8.**

KEYWORDS: gastric carcinoma, paclitaxel, proliferating cell nuclear antigen, flow cytometry.

Gastric cancer is one of the most common cancers in Asia. In Taiwan, gastric carcinoma is the third leading cause of death in male cancer patients and the sixth for females in 1993.[1] The outcome of patients with gastric carcinoma has recently been significantly improved due to advances in early diagnosis and surgical techniques.[2] Although chemotherapy[3] and radiation therapy[4] have been tried in either adjuvant or palliative treatments, their values are limited due to toxicity or the lack of efficiency.

Paclitaxel, isolated from the bark of the yew tree, is one of the antimitotic drugs used for treatment of various human cancers. Although the antitumor effect of paclitaxel has been known since 1971,[5] its clinical application has been delayed because of side effects. Paclitaxel inhibits tumor cell division by its action on microtubule assembly.[6] In vitro analysis using tumor cells has revealed that paclitaxel arrests cells not only in the G_2/M phases of cell cycle[7] but also in the G_0/G_1 phases.[8] Recent studies have shown that paclitaxel is effective against various malignant tumor

cells[9,10] such as breast cancer,[11] melanoma,[12] ovarian cancer,[13] brain tumor,[14] and prostate cancer.[15,16] Whether paclitaxel is effective against gastric carcinoma is not known and is worthy of investigation.

The objective of this study was to examine whether paclitaxel is effective in growth inhibition of human gastric carcinoma cells. The paclitaxel-induced effects examined included viability, morphologic change, cell growth inhibition, cell cycle progression, and proliferating cell nuclear antigen (PCNA) expression.

MATERIALS AND METHODS

Cell Culture

NUGC-3[17] and SC-M1[18] human gastric carcinoma cell lines, derived from Japanese and Chinese gastric carcinoma patients, respectively, were used. NUGC-3 cells were cultured in RPMI-1640 (GIBCO, Grand Island, NY) and Dulbecco's modified Eagle's medium (DMEM; GIBCO) in a 1:1 ratio and supplemented with L-glutamine (2 mM; GIBCO), kanamycin (100 μg/ml; GIBCO), amphotericin (1 μg/ml; GIBCO), and 10% fetal bovine serum (Hyclone, Logan, Utah). SC-M1 cells were cultured in RPMI-1640 with 10% fetal bovine serum and 0.01 mg/ml gentamycin (GIBCO). Cells were kept in a humidified incubator with 5% CO_2 and 95% air at 37°C.

Treatment

To examine the effect of paclitaxel on the in vitro growth of NUGC-3 and SC-M1 cells, paclitaxel was dissolved in dimethylsulfoxide (DMSO) and added to the culture media at various concentrations of 0.001, 0.01, 0.1, and 1 μM for 1–3 days. The final concentration of DMSO, which was used as the vehicle control, in the medium was 0.17%. Cells were counted by hemocytometer and cell viability was determined by the trypan blue exclusion method. Cells were harvested for flow cytometric analysis of cell cycle phase distribution and the expression of PCNA. In addition, the paclitaxel-treated and control cells were also collected for examination of apoptosis at Days 1 and 3.

Preparation of Cells for Flow Cytometry

The method of Landberg and Roos[19] was used. Briefly, cells harvested for flow cytometric analysis were washed twice with phosphate-buffered saline (PBS), and the cell pellet was then added with 0.5 ml of lysing buffer containing 0.5% Triton X-100, 0.2 μg/ml EDTA, and 1% bovine serum albumin in PBS. Cells were kept on ice for 15 minutes, followed by the addition of 3 ml methanol precooled at −20°C and left at room temperature for 10 minutes. After fixation, cells were washed twice with PBS and the cell pellet incubated with 10 ml fluorescein isothiocyanate (FITC)-conjugated monoclonal antibodies for PCNA (DAKO, PC-10, Copenhagen, Denmark) and 200 ml DNA staining medium [50 μg/ml propidium iodide, and 5 Kunitz/ml of RNase A (Sigma, St. Louis, MO) for 30 minutes at 4°C in the dark. PBS and IgG_{2a} were used as the negative and isotype controls, respectively. This was followed by washing with PBS twice. Finally, cells were resuspended in 0.3 ml PBS and analyzed on a flow cytometer.

TABLE 1
Effect of Paclitaxel on the Viability of SC-M1 and NUGC-3 Cells[a]

Group	Time of treatment (hr) 24	48	72
DMSO			
SC-M1 cells	96.52 ± 0.49	97.28 ± 0.19	96.35 ± 1.04
NUGC-3 cells	93.49 ± 1.06	88.52 ± 2.63	87.58 ± 3.81
Paclitaxel 0.001 μM			
SC-M1 cells	96.45 ± 0.42	96.12 ± 0.52	96.32 ± 0.04
NUGC-3 cells	90.44 ± 0.02	78.61 ± 8.99	89.70 ± 0.51
Paclitaxel 0.01 μM			
SC-M1 cells	95.16 ± 0.56	86.67 ± 1.90*	75.24 ± 1.76*
NUGC-3 cells	87.26 ± 1.59	62.55 ± 8.22	53.72 ± 0.65
Paclitaxel 0.1 μM			
SC-M1 cells	90.90 ± 0.35*	69.17 ± 0.20*	50.06 ± 2.30*
NUGC-3 cells	86.60 ± 0.57	43.75 ± 2.90	20.78 ± 1.27
Paclitaxel 1 μM			
SC-M1 cells	88.82 ± 1.81*	53.82 ± 0.94*	30.75 ± 0.08*
NUGC-3 cells	85.38 ± 0.91	36.24 ± 8.02	21.52 ± 2.16*

[a] Data are the percentage of viability (mean ± standard error, n = 3). Comparisons were between the treatment and DMSO control groups.
* Statistical significance at $P < 0.05$ using ANOVA.

Flow Cytometry

Cells (10,000) were analyzed on a Becton Dickinson (San Jose, California) FACScan flow cytometer using an argon-ion laser (15 mW) with the incident beam at 488 nm. Green fluorescence (FITC) corresponding to PCNA was collected through a 530-nm filter, and red fluorescence (propidium iodide) was collected through a 585-nm filter. The data were analyzed using a LYSYS II software on a HP-310 computer. Each experiment was repeated four times.

Staining

Cells were fixed on gelatin-coated slides using Cytospin (Shandon, Cheshire, UK) and stained with hematoxylin and eosin, then dehydrated through graded ethanol, cleared with xylene, mounted and examined. The percentages of cells with chromatin condensation or fragmented nuclei were calculated and compared between paclitaxel-treated and control cells.

Statistics

Comparisons between the paclitaxel-treated and DMSO control groups were determined using ANOVA. Differ-

ences between the means were considered as statistically significant at $P < 0.05$.

RESULTS

Table 1 shows the effect of paclitaxel on the viability of SC-M1 and NUGC-3 cells. The percentage of viable cells decreased significantly ($P < 0.05$) in a time- and dose-dependent manner for SC-M1 cells. A similar phenomenon was observed in NUGC-3 cells. A significant ($P < 0.05$) cytotoxic effect was observed after 72-hour treatment with paclitaxel at concentrations higher than 0.01 μM.

To examine further the effect of paclitaxel on the cell cycle progression of gastric carcinoma cells, DNA histograms of paclitaxel-treated and DMSO control cells were compared by flow cytometric analysis. Figure 1 shows that a low dose (0.001 μM) had no effect on the cell cycle progression up to 72 hours on both SC-M1 (Fig. 1A) and NUGC-3 cells (Fig. 1B). Treatment of both NUGC-3 and SC-M1 cells with 0.01 μM paclitaxel for 24 hours resulted in markedly reduced cells at G_0/G_1 and a concomitant increase in the typical apoptotic cells with decreased DNA content. The number of cells at G_2/M was not increased. Treatment of NUGC-3 and SC-M1 cells with 0.1 and 1.0 μM of paclitaxel for 24 hours resulted in an increase of cells at G_2/M. Continuous exposure of NUGC-3 and SC-M1 cells to 0.01–1 μM of paclitaxel for 72 hours resulted in a population of apoptotic cells with decreased DNA content.

Staining of the paclitaxel-treated and DMSO control cells showed that a significant increase in chromatin condensation or nuclear fragmentation was evident in 0.01–1.0 μM paclitaxel-treated SC-M1 and NUGC-3 cells (Table 2). At 24 hours after paclitaxel treatment most of the SC-M1 cells were chromatin condensed compared with control, whereas at 72 hours after paclitaxel treatment, most of the cells had membrane blebbing or nuclear fragmentation (Fig. 2A) compared with controls (Fig. 2B). Similar paclitaxel-induced changes were also found in NUGC-3 cells. These observations confirmed that these were apoptotic cells.

Since paclitaxel-induced DNA fragmentation in tumor cells[8] and PCNA is known to be involved in DNA repair,[20] we examined the effect of paclitaxel treatment on PCNA expression. Table 3 shows that treatment of SC-M1 and NUGC-3 cells with 0.1 and 1.0 μM paclitaxel for 72 hours resulted in a significant increase in the expression of PCNA.

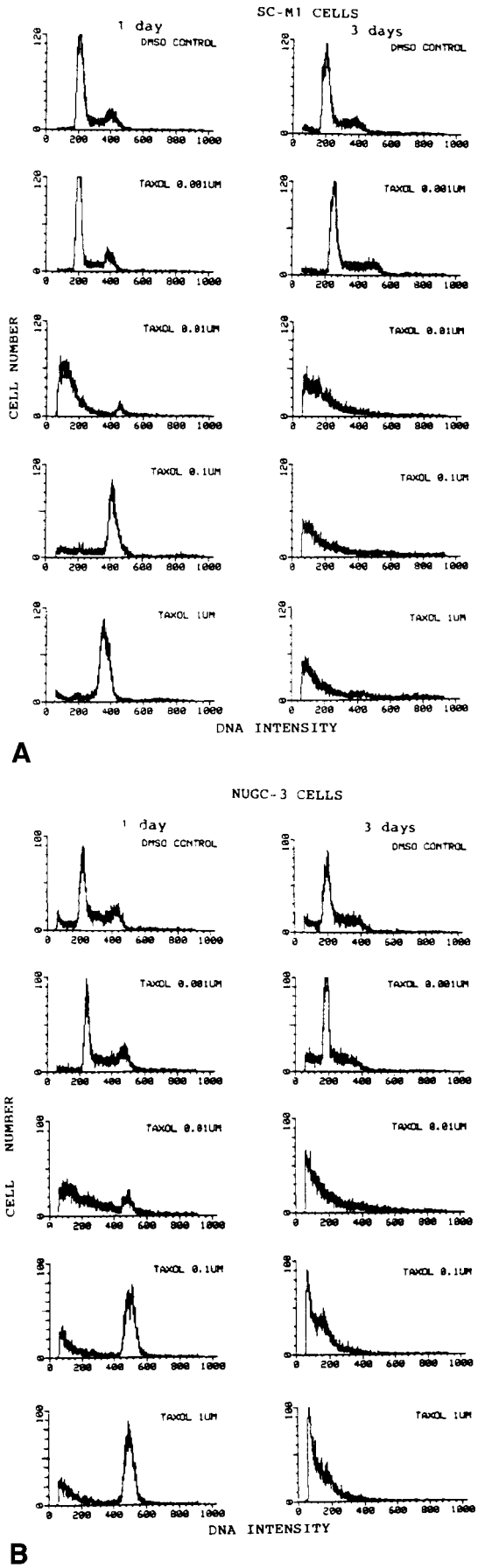

FIGURE 1. Effect of paclitaxel on the cell cycle progression of SC-M1 (A) and NUGC-3 (B) cells after a 24-hour or 72-hour treatment.

TABLE 2
Percentage of Paclitaxel-Induced Chromatin Condensation or Nuclear Fragmentation in SC-M1 and NUGC-3 Cells

	Time of treatment (hr)	
Group	24	72
DMSO		
SC-M1 cells	2.53 ± 0.47	1.28 ± 0.34
NUGC-3 cells	1.98 ± 0.20	1.92 ± 0.19
Paclitaxel 0.001 μM		
SC-M1 cells	4.97 ± 0.16	1.23 ± 0.21
NUGC-3 cells	1.97 ± 0.11	4.86 ± 0.24
Paclitaxel 0.01 μM		
SC-M1 cells	6.39 ± 0.33	12.15 ± 1.18*
NUGC-3 cells	9.37 ± 0.45*	13.92 ± 1.19*
Paclitaxel 0.1 μM		
SC-M1 cells	38.60 ± 0.78*	26.08 ± 1.66*
NUGC-3 cells	31.58 ± 0.57*	33.58 ± 2.28*
Paclitaxel 1 μM		
SC-M1 cells	38.22 ± 2.54*	29.56 ± 1.40*
NUGC-3 cells	44.48 ± 1.66*	38.07 ± 1.80*

[a] Data are the percentage of apoptotic cells (mean ± standard error, n = 3). Comparisons were between the treatment and DMSO control groups.
* Statistical significance at $P < 0.05$ using ANOVA.

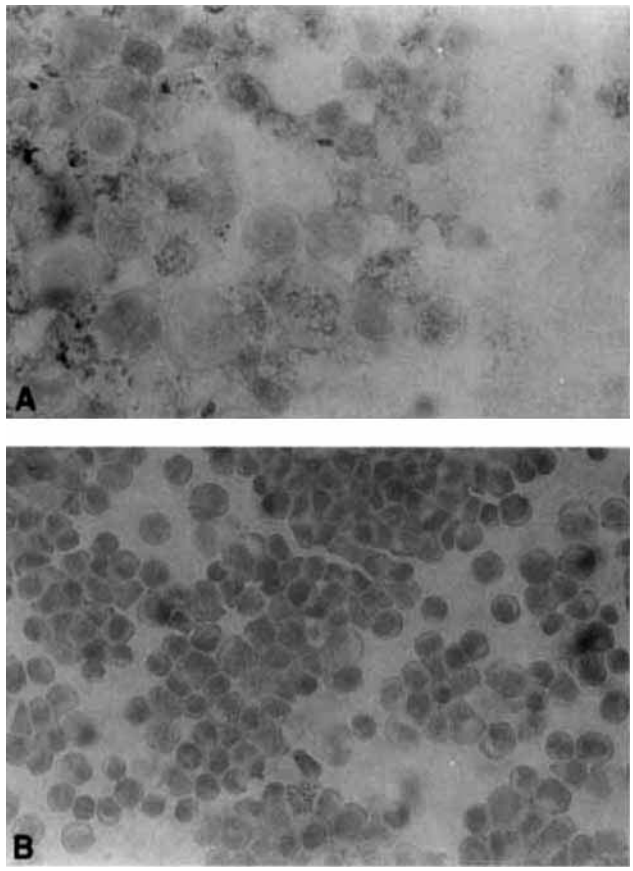

FIGURE 2. (A) SC-M1 cells treated with 1 μM paclitaxel for 72 hours. (B) DMSO-treated control cells. (Hematoxylin and eosin, original magnification ×400.)

DISCUSSION

The findings in this study show that paclitaxel has a cytotoxic effect on human gastric carcinoma lines in a dose- and time-dependent manner. A similar effect has been reported in breast,[11] ovary,[13] lung, and colon cancer[21] with an effective concentration of paclitaxel at 1 nM. However, in this study, paclitaxel at 1 nM had no effect on cell growth, viability, and cell cycle progression of gastric carcinoma cell lines for up to 72 hours of treatment.

In the present study, exposure of cells to 10 nM of paclitaxel for 24 hours appears to be cytotoxic. These observations are in agreement with the findings of Vanhoefer et al[22] who reported an IC50 of 37.0 nM for HM2 and 18.0 nM for HM15 gastric carcinoma cell lines after a 24-hour exposure to paclitaxel.

In this study it is interesting that paclitaxel at 10 nM acted mainly on cells in the G_0/G_1 phases, whereas at higher doses (0.1–1.0 μM) cells were arrested at G_2/M (Fig. 1A,B) before apoptosis. It is possible that the cytotoxic effect of paclitaxel on gastric carcinoma cells varies with drug concentration. The detailed mechanism of this differential paclitaxel-induced cytotoxic effect is not clear. Cells in paclitaxel-induced G_2/M arrest entered apoptosis with fragmented nuclei and an increased expression of PCNA (Table 3). By contrast, 10 nM paclitaxel treatment of gastric carcinoma cells for 24 hours resulted in apoptotic cells without G_2/M arrest or an increase in PCNA expression. These results suggest that the paclitaxel-induced cytotoxic effect is biphasic. High dose paclitaxel treatment resulted in DNA fragmentation, G_2/M arrest, and DNA repair. Low dose paclitaxel resulted in apoptosis of gastric carcinoma cells through an as yet unknown process that did not involve G_2/M arrest and DNA repair. This observation agrees well with Tomei et al.'s[23] findings that some cells may enter apoptosis without DNA fragmentation.

Using flow cytometry, we found that paclitaxel arrested cells mainly at the G_2/M phase (Fig. 1A,B). Similar effects have been reported in human breast and lung cancer cells[24] as well as in mouse fibroblast.[7] The observation of an accumulation of apoptotic cells (cells with less DNA content than G_0/G_1 cells) after paclitaxel treatment confirmed our observation that paclitaxel-induced cytotoxicity resulted in cell apoptosis.

A unique observation in this study is that paclitaxel treatment of gastric carcinoma cells resulted in increased expression of PCNA. A similar increase in the expression of PCNA has been reported in the drug-induced apoptotic leukemic cell line HL-60.[25] However, paclitaxel-treated gastric cells did not reenter the cell cycle (Fig. 1), as did

TABLE 3
Effect of Paclitaxel on the PCNA Intensity (mean channel) of SC-M1 and NUGC-3 Cells

Group	Time of treatment (hr)	
	24	72
DMSO		
SC-M1 cells	100	100
NUGC-3 cells	100	100
Paclitaxel 0.001 μM		
SC-M1 cells	74.6 ± 32.4	162.7 ± 4.7
NUGC-3 cells	92.4 ± 10.1	77.3 ± 20.7
Paclitaxel 0.01 μM		
SC-M1 cells	59.3 ± 20.8	146.8 ± 34.3
NUGC-3 cells	58.0 ± 23.1	103.9 ± 18.1
Paclitaxel 0.1 μM		
SC-M1 cells	64.9 ± 0.0	253.1 ± 0.1*
NUGC-3 cells	103.6 ± 10.1	321.0 ± 36.1*
Paclitaxel 1 μM		
SC-M1 cells	42.8 ± 5.9	296.9 ± 1.2*
NUGC-3 cells	98.1 ± 0.9	336.4 ± 11.9*

[1] Data are the percentage of the mean channel of DMSO group (mean ± standard error, n = 4). Comparisons were between the treatment and DMSO control groups.
* Statistical significance at $P < 0.05$ using ANOVA.

apoptotic leukemic cells. Therefore, the increased expression of PCNA was most likely involved in DNA repair rather than in DNA synthesis of paclitaxel-treated gastric carcinoma cells.

In conclusion, paclitaxel is effective in growth inhibition of gastric carcinoma cells in clinically attainable concentrations. Our results indicate that it has great potential for the treatment of human gastric carcinoma.

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