Paclitaxel (Taxol®) Inhibits Motility of Paclitaxel-resistant Human Ovarian Carcinoma Cells[1]

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ABSTRACT

The effect of paclitaxel on the adhesive and motility properties of human ovarian carcinoma cell lines was investigated. Paclitaxel significantly inhibited the motility of OVCAR 5, SK-OV-3, and HOC-1OTC ovarian carcinoma cell lines ($IC_{50} = 2.1 \times 10^{-8}$, 2×10^{-9}, and 1.9×10^{-8} M, respectively) but did not affect the adhesion of these cells to the subendothelial matrix. The association between inhibition of motility and cytotoxic activity was investigated using an A2780 subclone (1A9) and three paclitaxel-resistant variants (designated 1A9/PTX22, 1A9/PTX10, and 1A9/PTX18). Although paclitaxel did not significantly affect the adhesion to subendothelial matrix of the sublines, it completely inhibited their migration. Inhibition of migration was similar in 1A9 cells and the resistant sublines, with an IC_{50} of 1×10^{-8} for 1A9 cells and 5.4×10^{-9}, 1.1×10^{-8}, and 5.2×10^{-9} M for 1A9/PTX22, 1A9/PTX10, and 1A9/PTX18, respectively. Paclitaxel inhibited motility induced by soluble attractant (chemotaxis) and immobilized attractant (haptotaxis). Inhibition of cell motility occurred in the absence of an antiproliferative effect, because higher concentrations of paclitaxel were required to inhibit tumor cell proliferation (IC_{50} = 1.9×10^{-7} and 4.6×10^{-6}, 1×10^{-5}, and 3.1×10^{-6} M for 1A9 and 1A9/PTX22, 1A9/PTX10, and 1A9/PTX18, respectively). These data show that paclitaxel is a potent inhibitor of ovarian carcinoma cell motility and that this activity is independent of its cytotoxic activity.

INTRODUCTION

Paclitaxel (Taxol®), the first taxene to be introduced in clinical trials, is active against a broad spectrum of cancers, including those refractory to conventional chemotherapy (1). The mechanism of action of paclitaxel is to favor polymerization and stabilization of microtubules (2). Although the main function of microtubules is the formation of the mitotic spindle during cell division, they are also involved in several other vital functions, including the maintenance of cell shape, adhesion, motility, and signal transduction. It has been reported that drugs that inhibit the polymerization of microtubules also inhibit cell migration and invasiveness (3, 4).

Since its initial approval for treating cisplatin-resistant ovarian cancer and the encouraging preliminary results in women with advanced diseases, substantial antitumor activity of paclitaxel in patients with ovarian cancer has been reported (5–8). Paclitaxel is proposed currently as a first-line treatment for this neoplasm.

Spreading of ovarian cancer occurs initially throughout the abdominal cavity, with ascites formation and involvement of abdominal organs (9). Under these circumstances, adhesion and migration of ovarian carcinoma cells in the peritoneal cavity play a critical role in the dissemination of the disease (10, 11).

The aim of this study was to investigate the effect of paclitaxel on adhesion and motility of human ovarian carcinoma cells. We show that paclitaxel affects the migration, but not the adhesion, of several human carcinoma cell lines. Using paclitaxel-resistant ovarian carcinoma sublines, we demonstrate that inhibition of cell motility is not directly associated with the antiproliferative activity of paclitaxel.

MATERIALS AND METHODS

Drugs. Paclitaxel was provided by Bristol-Myers Squibb, (Princeton, NJ). A 1000× stock solution in absolute ethanol was prepared freshly and diluted in the test medium before each experiment. Control medium contained an equal volume of absolute ethanol.

Cells. The human ovarian carcinoma lines OVCAR 5 and SK-OV-3 were obtained from the Division of Cancer Treatment, Tumor Repository (National Cancer Institute, Frederick, MD). The HOC-10TC was established in culture from the ascites of a patient with ovarian carcinoma (12).

The 1A9 cell line is a subclone of the A2780 human ovarian carcinoma cell line. Three paclitaxel-resistant sublines designated 1A9/PTX22, 1A9/PTX10, and 1A9/PTX18 were derived from the 1A9 cells and will be described elsewhere.[3] Briefly, the resistant sublines were isolated as single clones after an initial exposure to 5 ng/ml paclitaxel in the presence of 5 μg/ml verapamil. After the initial isolation, the concentration of paclitaxel was advanced incrementally to a concentration of 30 ng/ml. The 1A9/PTX22, 1A9/PTX10, and 1A9/PTX18 sublines were, respectively, 24-, 53-, and 16-fold more resistant to pa-

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clitaxel than 1A9 parental cells, as calculated from the ratio of the IC_{50} of the resistant and parental cells. Verapamil was included to preclude the isolation of cell lines expressing P-glycoprotein, and neither the parental cells nor the three drug-resistant sublines express MDR-1, as determined by PCR. *In vitro* studies with live cells and with purified tubulin have shown that the addition of paclitaxel does not lead to tubulin polymerization in the resistant sublines even at concentrations that are as much as 100-fold greater than the cytotoxic concentrations. The resistant phenotype has been shown to be stable for more than 2 years in cells maintained without paclitaxel.

Tumor cell lines were grown as monolayers in RPMI supplemented with 10% FCS (Life Technologies, Inc., Paisley, Scotland). The paclitaxel-resistant sublines were maintained in culture medium supplemented with paclitaxel (30 ng/ml) and verapamil (5 μg/ml). To exclude an effect of continuous paclitaxel exposure on the assays performed, cells were grown in paclitaxel-free, verapamil-free medium for 1 week prior to any assay. Verapamil was usually not present in the test medium; however, in our initial experiments, we observed that the addition of 5 μg/ml verapamil did not affect cell motility nor the inhibitory effect of paclitaxel on motility.

Bovine aortic endothelial cells were provided kindly by E. Dejana (Mario Negri Institute, Milan, Italy) and maintained in DMEM with 10% FCS.

Proliferation Assay. Tumor cells were plated in a 96-well plate in culture medium (5×10^3/0.1 ml/well). After 24 h, fresh medium containing paclitaxel (1×10^{-11}–1×10^{-5} M) was added for 4 h. Cells were washed and incubated for an additional 72 h and then fixed and stained with 0.5% crystal violet in 20% methanol, rinsed, and air dried. The stain was eluted with a 1:1 solution of ethanol 0.1 M sodium citrate, and absorbance at 540 nm was measured with a Multiscan MC (Titertek; Flow Laboratories, Milan, Italy). Data are expressed as a percentage of the control (samples containing the same amount of absolute ethanol). The amount of drug that caused 50% inhibition of cell growth (IC_{50}) was calculated from the plotted data.

To evaluate the cytotoxic effect of paclitaxel in experimental conditions comparable to the motility assays (see below), tumor cell suspensions were exposed to different concentrations of paclitaxel in RPMI with O.1% BSA for 4 h at 37°C. Tumor cells were then washed and plated in culture medium in a 96-well plate, and after 3 days, the cells were fixed and stained as described above.

Motility Assays. Cell motility was assayed using modified Boyden chambers with 8 μm-pore size polycarbonate polyvinylpyrrolidone-free Nucleopore filters, as described (13). Filters were coated with gelatin by immersing them overnight in a solution of 100 μg/ml gelatin in 0.1% acetic acid and air dried. Among several chemoattractants, the NIH-3T3-conditioned medium was chosen as the most active attractant for all the ovarian tumor cell lines. To test the motility response to matrix, we used laminin, purified from the EHS murine sarcoma (14), as either chemotactic and haptotactic attractant. For chemotaxis, laminin (20 μg/ml) was added to the lower compartment of the Boyden chamber; for haptotaxis, the side of the filter facing the lower compartment was coated with laminin by floating it overnight on a solution of 20 μg/ml laminin in PBS at 37°C, as described previously (13). Cells were detached by brief exposure to 0.25% trypsin-0.02% EDTA, washed with RPMI supplemented with 0.1% BSA, resuspended in this medium at the final concentration of 2×10^6/ml, and added to the upper compartment of the chamber. Paclitaxel (10^{-10}–10^{-5} M) was added to the cell suspension and left during the assay (4 h). After a 4-h incubation at 37°C, filters were stained with Diff-Quick (Marz-Dade, Dudingen, Switzerland), and the migrated cells in 20 high-power fields were counted. Each sample was tested at least in triplicate. Results are expressed as the percentage of control motility (migration in the presence of vehicle), and IC_{50} (dose causing 50% inhibition) was calculated from the plotted data.

Adhesion Assay. Subendothelial matrix was prepared as described previously by dissolving a layer of confluent bovine endothelial cells, grown in a 96-well plate, with a solution of 0.5 Triton X-100 and 20 mM NH_4OH in Dulbecco's PBS, at room temperature for 3 min, followed by four washes in Dulbecco's PBS (14). Nonspecific binding sites were saturated by a 30-min incubation with 1% BSA in serum-free medium. Tumor cells in serum-free medium containing 0.1% BSA were incubated with paclitaxel for 4 h and then added to the plate (5×10^4 cells/well). After 15 min at 37°C, the wells were washed gently three times with serum-free medium. Adherent cells were fixed and stained with 0.5% crystal violet in 20% methanol, rinsed with water, and air dried. The stain was then eluted and absorbance read at 540 nm, as described for the proliferation assay. In some experiments, paclitaxel (10^{-10} M to 10^{-5} M) was added to already attached tumor cells and left for 4 h. Data are expressed as a percentage of adherent cells compared to control (vehicle-treated cells).

RESULTS

Effect of Paclitaxel on Adhesion and Motility of Human Ovarian Carcinoma Cell Lines. To evaluate the effect of paclitaxel on the adherence of ovarian carcinoma cell lines to the subendothelial extracellular matrix, tumor cells were exposed to different concentrations of paclitaxel for 4 h before the adhesion assay. In these conditions, paclitaxel (up to 10^{-5} M) did not affect the adhesion of OVCAR 5, SK-OV-3, and HOC-10TC (Fig. 1).

Motility was measured in a Boyden chamber, in response to NIH-3T3-conditioned medium. The exposure of cells to paclitaxel during the 4-h assay inhibited the motility response of all the cell lines (Fig. 1), with average IC_{50}s of 2.1×10^{-8}, 2×10^{-9}, and 1.9×10^{-8} M for OVCAR 5, SK-OV-3, and HOC-10TC cells, respectively (Table 1).

The antiproliferative and antimigratory effects of paclitaxel on ovarian carcinoma cell lines are summarized in Table 1.

Effect of Paclitaxel on Adhesion, Motility, and Proliferation of Paclitaxel-resistant Ovarian Carcinoma Sublines. To determine the inhibitory activity of paclitaxel on drug-resistant tumor cells, we compared the effect of paclitaxel on the adhesion, motility, and proliferation of parental 1A9 cells and three paclitaxel-resistant sublines. Parental 1A9 cells and the three 1A9/PTX-resistant sublines (1A9/PTX22, 1A9/PTX10, and 1A9/PTX18) provide a suitable model to compare these effects of paclitaxel. Both sensitive and resistant cell lines showed a similar degree of adhesion to the extracellular matrix

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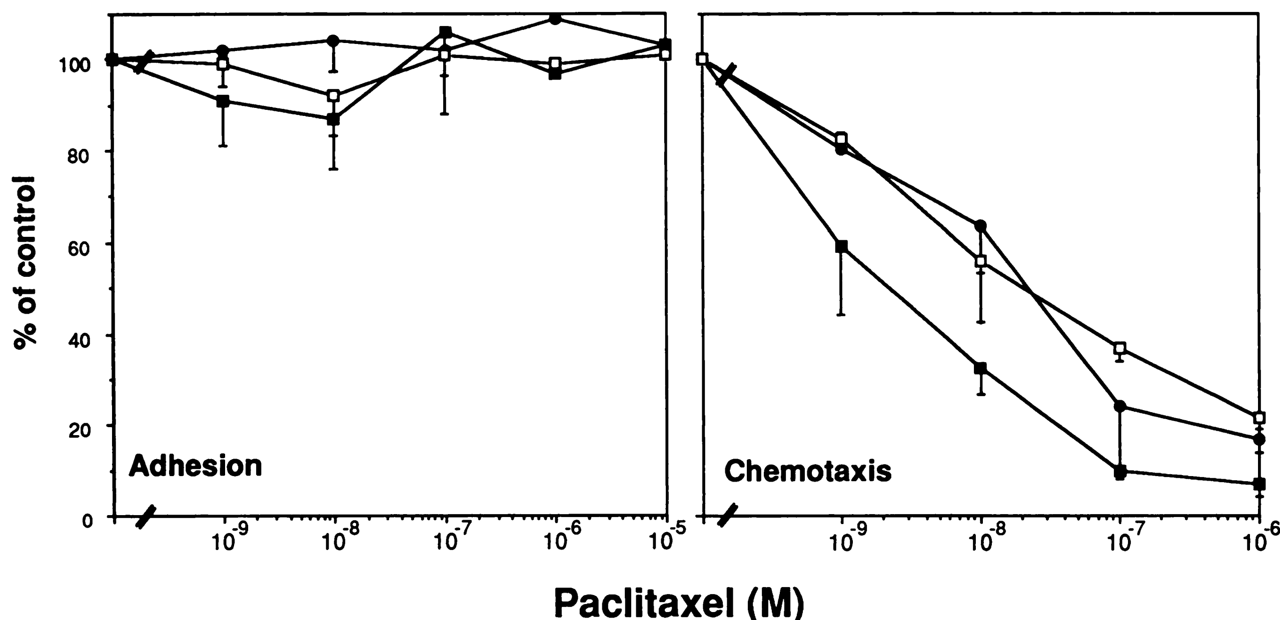

Fig. 1 Effect of paclitaxel on adhesion and migration of the human ovarian carcinoma cell lines. For the adhesion assay, cells were exposed to different concentrations of paclitaxel for 4 h and maintained in drug throughout the assay. Adhesion of vehicle-treated cells to subendothelial matrix, expressed as absorbance at 540 nm, was 1.49 ± 0.09 for OVCAR 5, 0.56 ± 0.06 for SK-OV-3, and 0.56 ± 0.04 for HOC-10TC. For migration, cells were exposed to different concentrations of paclitaxel throughout the 4-h chemotaxis assay. Vehicle-treated migrated cells, counted in 20 high-power fields, were 22.0 ± 0.3 for OVCAR 5, 57.5 ± 2.7 for SK-OV-3, and 19.2 ± 1.4 for HOC-10TC. Data are expressed as a percentage of vehicle-treated controls (means and SEs of triplicate data) and are from one experiment that is representative of at least two. ●, OVCAR 5; ■, SK-OV-3; □, HOC-10TC .

Table 1 Comparison of the antiproliferative and antimigratory effect of paclitaxel on human ovarian carcinoma cell lines[a]

	IC_{50} (M)	
Tumor line	Proliferation	Motility
OVCAR 5	2.4×10^{-7}	2.1×10^{-8}
SK-OV-3	8.4×10^{-7}	2.0×10^{-9}
HOC-10TC	$>10^{-6}$	1.9×10^{-8}
1A9	1.9×10^{-7}	1.0×10^{-8}
1A9/PTX22	4.6×10^{-6}	5.4×10^{-9}
1A9/PTX10	1.0×10^{-5}	1.1×10^{-8}
1A9/PTX18	3.1×10^{-6}	5.2×10^{-9}

[a] Tumor cells were exposed to paclitaxel for 4 h in both the assays. Data are expressed as IC_{50}, the concentration of paclitaxel causing 50% inhibition. Results are the average values of data from at least two independent experiments.

(ranging from a 3.7- to a 6.1-fold increment of adhesion compared to uncoated plastic for the four sublines) and a chemotactic response to NIH-3T3-conditioned medium (28–52 migrated cells in 20 high power fields). Moreover, the acquisition of paclitaxel resistance only marginally affected the *in vitro* growth rate of tumor cells (mean doubling times were 29–34 h).

Adhesion. Paclitaxel did not significantly affect the adherence of all the sublines even at high concentration (up to 10^{-5} M; Fig. 2). A marginal inhibition (never exceeding 20%) was observed in different experiments independent of drug concentration. The effect of paclitaxel on the adhesion of 1A9 and 1A9/PTX22 cells to purified extracellular matrix components (laminin, type IV collagen, and thrombospondin) was assayed. Also on these substrates, adhesion was not significantly affected by paclitaxel, even at the dose of 10^{-5} M (data not shown).

To verify whether paclitaxel might detach tumor cells adherent to a substrate, 1A9 and 1A9/PTX22 tumor cells, already attached to the subendothelial matrix, were treated with different concentrations of paclitaxel for 4 h. Even at high concentrations, paclitaxel did not affect tumor cell attachment (5.6 and 4.5% reduction in cell attachment for subline 1A9 and 1A9/PTX22, respectively, at 10^{-5} M paclitaxel). Similar results were obtained when cells adherent to tissue culture plastic were exposed to paclitaxel (data not shown).

Motility. Paclitaxel completely inhibited the motility response of 1A9 cells and the 1A9/PTX-resistant sublines to NIH-3T3-conditioned medium. Fig. 2 shows that the inhibition was dose dependent beginning at concentrations above 10^{-9} M and reaching a complete inhibition at doses $\geq 10^{-7}$. No significant difference in the extent of inhibition was observed between 1A9 and the paclitaxel-resistant 1A9/PTX sublines, at all the concentrations. The drug concentration that caused 50% inhibition of cell motility was similar among the parental and resistant cell lines: average IC_{50} was 1×10^{-8} M for parental 1A9 cells, and 5.4×10^{-9}, 1.1×10^{-8}, and 5.2×10^{-9} M for the resistant sublines 1A9/PTX22, 1A9/PTX10, and 1A9/PTX18, respectively (Table 1). The presence of paclitaxel during the assay was not required for inhibition. Inhibition of chemotaxis was also observed when cells, preincubated with paclitaxel for 4 h, had

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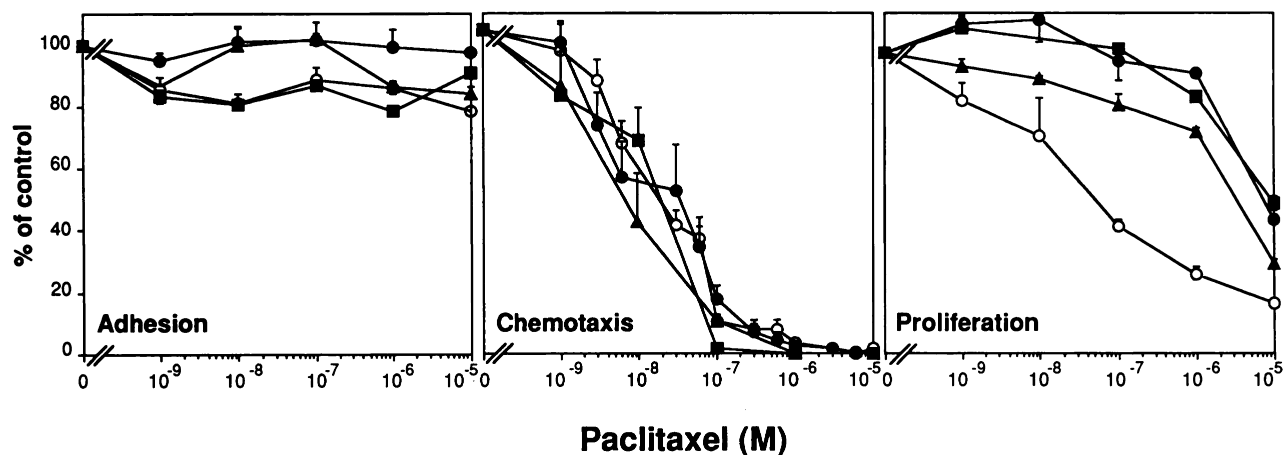

Fig. 2 Effect of paclitaxel on adhesion, migration, and proliferation of parental 1A9 cells and three paclitaxel-resistant sublines. Adhesion to subendothelial matrix and migration to NIH-3T3-conditioned medium were assessed as described in the legend to Fig. 1. Adhesion of vehicle-treated cells, expressed as absorbance at 540 nm, was 0.57 ± 0.01 for 1A9 cells and 1.11 ± 0.02, 0.66 ± 0.02, and 0.62 ± 0.02 for 1A9/PTX22, 1A9/PTX10, and 1A9/PTX18, respectively. The number of vehicle-treated migrated cells, counted in 20 high-power fields, was 39.5 ± 4.9 for 1A9 and 52.0 ± 3.3, 28.3 ± 4.6, and 48.5 ± 3.0, for 1A9/PTX22, 1A9/PTX10 and 1A9/PTX18, respectively. Proliferation was assayed as described in "Materials and Methods" after a 4-h exposure to different concentrations of paclitaxel. Data are expressed as a percentage of vehicle-treated controls (means and SEs of triplicate data), and are from one experiment that is representative of two to six. ○, 1A9; ●, 1A9/PTX22; ▲, 1A9/PTX18; ■, 1A9/PTX10.

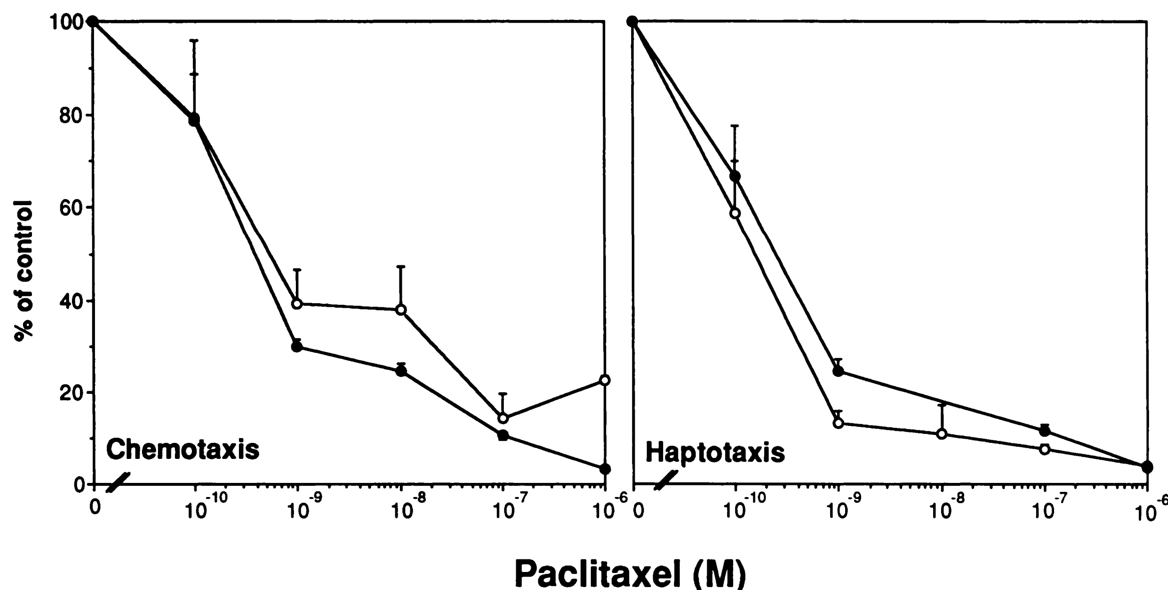

Fig. 3 Effect of paclitaxel on chemotaxis and haptotaxis of 1A9 and 1A9/PTX22 cells to laminin. Chemotaxis (motility induced by soluble laminin) and haptotaxis (motility induced by substrate-bound laminin) were conducted as described in "Materials and Methods." 1A9 (○) and 1A9/PTX22 (●) were exposed to increasing concentrations of paclitaxel throughout the assay. Data are expressed as a percentage of vehicle-treated controls (means and SEs of triplicate data) and are from one experiment that is representative of at least two.

paclitaxel removed before the assay (89.6 and 79.3% inhibition, respectively, for 1A9 and 1A9/PTX22 at 10^{-6} M paclitaxel).

Paclitaxel also inhibited cell migration to the matrix component laminin. Paclitaxel completely inhibited the chemotactic (migration to soluble laminin) and haptotactic response (migration to substrate-bound laminin) of the parental 1A9 and the resistant 1A9/PTX22 cells (Fig. 3). Inhibition of chemotaxis and haptotaxis occurred at similar concentrations of paclitaxel for both the parental and the resistant cell lines (Fig. 3).

Proliferation. Because the motility of parental and resistant sublines was affected equally, we investigated whether the inhibition of motility was linked to a direct cytotoxic effect of paclitaxel. Fig. 2 and Table 1 show the effect of a 4-h paclitaxel treatment on cell proliferation. Paclitaxel was 16- to

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53-fold more toxic to parental 1A9 cells than to the three resistant 1A9/PTX sublines; and for all the cell lines, the concentrations of paclitaxel that inhibited cell motility were lower than those required to affect proliferation. Even a longer exposure (72 h) to the concentrations of paclitaxel that affected cell motility (10^{-9}–10^{-8} M) inhibited proliferation of parental IA9 cells but not of the resistant 1A9/PTX sublines (data not shown).

To evaluate the cytotoxic effect of paclitaxel in experimental conditions similar to the chemotaxis assay, 1A9 and 1A9/PTX22 cell suspensions exposed to increasing concentrations of paclitaxel for 4 h were washed before plating for the proliferation assay. Under these conditions, 1A9/PTX22 cells maintained their resistance to paclitaxel compared to parental 1A9 cells. The IC_{50} was 4.1×10^{-8} M and 3.1×10^{-6} M for 1A9 and 1A9/PTX22, respectively (data not shown).

DISCUSSION

Adhesion and migration are two functions necessary for the spread of ovarian carcinoma cells in the peritoneal cavity. In the present study, we show that paclitaxel strongly inhibits motility in ovarian carcinoma cell lines independent of their sensitivity to the cytotoxic effect of the drug. In contrast, the adhesion of ovarian carcinoma cells to extracellular matrix was unaffected by paclitaxel.

Microtubule-affecting agents, such as colchicine and the *Vinca* alkaloids, have been shown to affect cell motility and related activities (3). Our evidence that paclitaxel inhibits the motility of ovarian cancer cells confirms and extends previous observations on the effect of this drug on the motility of fibroblasts and other tumor cell types (4, 15–17). One of these studies showed an effect of paclitaxel in proteinase production and secretion, tumor cell invasion, and metastasis, suggesting a role for this drug in preventing tumor spreading (16). However, these studies have not investigated the relationship between paclitaxel cytotoxicity and inhibition of motility.

In the present study, we observed inhibition of cell migration at lower paclitaxel concentrations (10–900-fold; Table 1) than those required to affect cell proliferation. This suggests that low paclitaxel concentrations that are unable to affect cell proliferation might inhibit tumor cell migration.

The concentrations of paclitaxel used in this study range from 10^{-9} to 10^{-5} M. Inhibition of cell migration is seen at concentrations of 10^{-9} and higher, and inhibition of cell proliferation at concentrations of 10^{-8} and higher, thus at concentrations well below the plasma concentrations that can be achieved in patients (18).

Furthermore, we show that paclitaxel inhibits the motility of parental and paclitaxel-resistant cells equally. Using the same experimental conditions, resistant cells were shown to be tolerant to paclitaxel cytotoxic effects as measured by cell proliferation, but sensitive to the effect of the drug on motility. We conclude that the effect of paclitaxel on cell motility is independent of its effect on cell proliferation. Furthermore, our results suggest that paclitaxel affects cell migration at lower drug concentrations than those required to affect cell proliferation. We speculate that paclitaxel has an effect on the cytoskeletal rearrangements necessary for the cell movement, which immediately follow the receptor-chemoattractant interaction (19). Because inhibition of motility was similar in both parental and paclitaxel-resistant cells, we hypothesize that, in our tumor model, resistance to the cytotoxic activity of paclitaxel does not necessarily confer resistance to the antimigratory effect of the drug. Preliminary studies indicate that the accumulation of paclitaxel is similar in both parental cells and the drug-resistant variants, which do not express P-glycoprotein. Instead, the mechanism of resistance appears to be mediated through changes in at least one tubulin isotype.[3]

Paclitaxel did not affect the adhesion of ovarian carcinoma cells to the subendothelial extracellular matrix. These results are in contrast with previous reports showing that paclitaxel inhibited the adhesion of prostatic and melanoma cells to plastic and to other adhesive substrata (15, 16). The difference in tumor cell types and substrate characteristics might account for this discrepancy. Interestingly, in one of these studies, a greater effect of paclitaxel on melanoma cell motility than on melanoma cell adhesion was reported (15). These authors also reported a strong inhibition of motility of preattached cells, suggesting that prevention of microtubule depolymerization by paclitaxel "freezes" the cell in a spread conformation, blocking motility (15). The lack of inhibition on ovarian carcinoma cell adhesion by paclitaxel might therefore be explained by the limited requirement for microtubule depolymerization in adhesion compared to motility.

Because paclitaxel did not inhibit cell adhesion to matrix, we studied its effect on cell migration induced by a matrix component, laminin. Extracellular matrix is known to induce motility through two different mechanisms: chemotaxis, motility induced by soluble attractant; or haptotaxis, motility in response to substrate-bound attractant, in which the attractant also provides the adhesive substrate. Paclitaxel inhibited both mechanisms of motility to laminin, to a similar extent for the parental line and the resistant subline, thus confirming that inhibition of motility by paclitaxel does not depend on the attractant nor on the mechanism of motility.

In conclusion, we have shown that paclitaxel is a strong inhibitor of ovarian carcinoma cell motility. Ovarian carcinoma spreads throughout the abdominal cavity, resulting in disseminated peritoneal implantation. This dissemination and the lack of complete response to chemotherapy are the main obstacles to the treatment of ovarian cancer. Blocking at least one activity of tumor cells essential to invade, such as migration, may make it possible to interfere with the dissemination process. The finding that paclitaxel inhibits the motility of resistant tumor cells might have clinical significance for the antineoplastic activity of paclitaxel, acting as both a cytotoxic and an anti-invasive agent.

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REFERENCES

1. Rowinsky, E. K., and Donehower, R. C. Paclitaxel (Taxol). N. Engl. J. Med, *332:* 1004–1014, 1995.

2. Jordan, M. A., Toso, R. J., Thrower, D., and Wilson, L. Mechanism of mitotic block and inhibition of cell proliferation by taxol at low concentration. Proc. Natl. Acad. Sci. USA, *90:* 9552–9556, 1993.

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3. Mareel, M. M., and De Mets, M. Effect of microtubule inhibitors on invasion and on related activities of tumor cells. Int. Rev. Cytol., *90:* 125–168, 1984.

4. Schiff, P. B., and Horwitz, S. B. Taxol stabilizes microtubules in mouse fibroblast cells. Proc. Natl. Acad. Sci. USA, *77:* 1561–1565, 1980.

5. Donehower, R. C., Rowinsky, E. K., Grochow, L. B., Longnecker, S. M., and Ettinger, D. S. Phase I trial of taxol in patients with advanced cancer. Cancer Treat. Rep., *71:* 1171–1177, 1987.

6. Caldas, C., and McGuire, W. P, III. Taxol in epithelial ovarian cancer. Monogr. Natl. Cancer Inst., *15:* 155–159, 1993.

7. McGuire, W. P., Rowinsky, E. K., Rosenshein, N. B., Grumbine, F. C., Ettinger, D. S., Armstrong, D. K., and Donehower, R. C. Taxol: a unique antineoplastic agent with significant activity in advanced ovarian epithelial neoplasms. Ann. Intern. Med., *111:* 273–279, 1989.

8. Einzig, A. I., Wiernik, P. H., Sasloff, J., Runowicz, C. D., and Goldberg, G. L. Phase II study and long-term follow-up of patients treated with Taxol for advanced ovarian adenocarcinoma. J. Clin. Oncol., *10:* 1748–1753, 1992.

9. Cannistra, S. A. Cancer of the ovary. N. Engl. J. Med., *329:* 1550–1559, 1993.

10. Cannistra, S. A., Kansas, G. S., Niloff, J., DeFranzo, B., Kim, Y., and Ottensmeier, C. Binding of ovarian cancer cells to peritoneal mesothelium *in vitro* is partly mediated by CD44H. Cancer Res., *53:* 3830–3838, 1993.

11. Liebman, J. M., Burbelo, P. D., Yamada, Y., Fridman, R., and Kleinman, H. K. Altered expression of basement-membrane components and collagenases in ascitic xenografts of OVCAR-3 ovarian cancer cells. Int. J. Cancer, *55:* 102–109, 1993.

12. Massazza, G., Tomasoni, A., Lucchini, V., Allavena, P., Erba, E., Colombo, N., Mantovani, A., D'Incalci, M., Mangioni, C., and Giavazzi, R. Intraperitoneal and subcutaneous xenografts of human ovarian carcinoma in nude mice and their potential in experimental therapy. Int. J. Cancer, *44:* 494–500, 1989.

13. Taraboletti, G., Belotti, D., Dejana, E., Mantovani, A., and Giavazzi, R. Endothelial cell migration and invasiveness are induced by soluble factor produced by murine endothelioma cells transformed by polyoma virus middle T oncogene. Cancer Res., *53:* 3812–3816, 1993.

14. Taraboletti, G., Belotti, D., Giavazzi, R., Sobel, M. E., and Castronovo, V. Enhancement of metastatic potential of murine and human melanoma cells by laminin receptor peptide G: attachment of cancer cells to subendothelial matrix as a pathway for hematogenous metastasis. J. Natl. Cancer Inst., *85:* 235–240, 1993.

15. Stracke, M. L., Soroush, M., Liotta, L. A., and Schiffmann, E. Cytoskeletal agents inhibit motility and adherence of human tumor cells. Kidney Int., *43:* 151–157, 1993.

16. Stearns, M. E., and Wang, M. Taxol blocks processes essential for prostate tumor cell (PC-3 ML) invasion and metastases. Cancer Res., *52:* 3776–3781, 1992.

17. Verschueren, H., Dewit, J., De Braekeleer, J., Schirrmacher, V., and De Baetselier, P. Motility and invasive potency of murine T-lymphoma cells: effect of microtubule inhibitors. Cell Biol. Int., *18:* 11–19, 1994.

18. Rowinsky, E. K., Cazenave, L. A., and Donehower, R. C. Taxol: a novel investigational antimicrotubule agent. J. Natl. Cancer Inst., *82:* 1247–1259, 1990.

19. Singer, S. J., and Kupfer, A. The directed migration of eukaryotic cells. Annu. Rev. Cell Biol., *2:* 337–365, 1986.

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