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**ORIGINAL ARTICLE**

# Comparison in purity and antitumor effect of brand and generic paclitaxel against human ovarian cancer cells by an in vitro experimental model

Kung-Liahng Wang[1,2,3], Yuh-Cheng Yang[1,4], Jerry Cheng-Yen Lai[4], Tung-Hu Tsai[5], Chin-Ping Lin[4], Yu-Tse Wu[5], Yu-Yawn Chen[6], Shen-Chuan Wang[2,7] and Yu-Jen Chen[4,8,9]

*[1]Department of Obstetrics and Gynecology, Mackay Memorial Hospital, Taipei, Taiwan, PR China, [2]National Taipei College of Nursing, Taipei, Taiwan, PR China, [3]Mackay Medicine, Nursing and Management College, Taipei, Taiwan, PR China, [4]Department of Medical Research, Mackay Memorial Hospital, Taipei, Taiwan, PR China, [5]Institute of Traditional Medicine, School of Medicine, National Yang-Ming University, Taipei, Taiwan, PR China, [6]Department and Graduate School of Physical Education, National Taiwan College of Physical Education, Taichung, Taiwan, PR China, [7]Department of Pharmacy, Mackay Memorial Hospital, Taipei, Taiwan, PR China, [8]Department of Radiation Oncology, Mackay Memorial Hospital, Taipei, Taiwan, PR China and [9]Graduate Institute of Sport Coaching Science, Chinese Culture University, Taipei, Taiwan, PR China*

**Abstract**

*Context*: The purity and the therapeutic effectiveness of the generic paclitaxel have not yet been examined and compared to the original brand form. *Objective*: This study aimed to compare the in vitro purity and biological effects of original brand form (Taxol) and a generic drug of paclitaxel. *Materials and Methods*: Purity was determined by high-performance liquid chromatography analysis, cell viability by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide assay, cell proliferation by clonogenic assay, morphology by Liu's staining, and cell cycle distribution by DNA histogram. *Results*: Taxol and generic paclitaxel shared similar high-performance liquid chromatography profiles with a major peak at the same retention time and ultraviolet spectrum. Generic paclitaxel inhibited the cell viability to an extent greater than Taxol. By assessing the $IC_{50}$, generic paclitaxel also exhibited a greater inhibitory activity on clonogenicity of human ovarian adenocarcinoma SKOV-3 cells. Although both generic paclitaxel and Taxol arrested SKOV-3 and ES-2 cells at G2/M phase with concurrent development of hypoploid and polyploid cells, Taxol treatment exhibited markedly less extent of these changes. Observation of cellular morphology revealed a greater amount of mitotic catastrophe-like and apoptotic cells in generic paclitaxel-treated cells than Taxol-treated cells. *Discussion and Conclusion*: The results suggest that generic paclitaxel may possess a greater cell death inducing capacity and clonogenicity inhibitory activity against ovarian cancer cells than the original brand Taxol of the same purity. We conclude that this experimental model for assessing the difference between generic and brand name drugs might be considered as a reference while determining their interchangeability and could be easily established in a hospital-based laboratory.

**Key words:** *Brand; generic; interchangeability; paclitaxel; taxol*

## Introduction

After expiration of patent protection of original brand, generic products are usually marketed at a lower price after drug approval. The interchangeability between a generic drug and the corresponding brand name drug is based on the principle of essential similarity, which includes the same component of active ingredients[1], the same route of administration, and the same therapeutic effectiveness[2]. To address the issues on bioavailability and bioequivalence, clinical trials[3] are considered, but not absolutely required, before the approval of generic

*Address for correspondence:* Dr. Yu-Jen Chen, MD, PhD, Department of Radiation Oncology, Mackay Memorial Hospital, 92, Section 2, Chung-Shan North Road, Taipei 10449, Taiwan, PR China. Tel: +886 228094661, Fax: +886 228096180. E-mail: chenmdphd@yahoo.com

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products, which depends on regulations in different countries.

Clinical registration studies on this purpose may take a great cost and extensively prolong the time before drug approval[4]. Clearly, for assessing the interchangeability, a less time-consuming and labor-intensive examination is critically needed. This need also refers to that while assessing the interchangeability between various generic drugs from different origins.

Paclitaxel is an effective chemotherapeutic drug against ovarian cancer[5]. The purity and the therapeutic effectiveness of the generic paclitaxel have not yet been examined and compared to the original brand one. The aim of this study was to establish an experimental model and compare the similarity between the original brand (Taxol) and the generic drugs of paclitaxel based on in vitro purity as well as bioactivity testing in a hospital-based laboratory.

## Materials and methods

### *Cell cultures*

The human ovarian adenocarcinoma SKOV-3 and the clear cell carcinoma ES-2 cells were obtained from American Type Culture Collection (Manassas, VA, USA). The cells were maintained in a culture using Dulbecco's modified eagle medium with 100 units/mL penicillin, 100 μg/mL streptomycin, 2 mM-glutamine (all from Invitrogen, Carlsbad, CA, USA), and 10% fetal bovine serum (Atlanta Biologicals, Norcross, GA, USA) at 37°C in a humidified 5% $CO_2$ atmosphere.

### *Chemical and reagents*

Taxol, the brand name paclitaxel, was purchased from Bristol–Myers Squibb Co. (Princeton, NJ, USA). A generic paclitaxel was purchased from Sinphar Pharmaceutical Co. (I-Lan, Taipei, Taiwan; and Phytogen Life Sciences Inc., Delta, Canada). Confluent cultures of cells were exposed to these drugs dissolved in dimethyl sulfoxide (DMSO). Control cells were exposed to DMSO only. The final concentration of DMSO was adjusted to less than 0.1% (v/v), a concentration without toxicity to these two tested cell lines determined in our preliminary work. Before subjected to experiments by research assistants, these drugs were randomly labeled by principal investigators to ensure a blinded performance. In some experiments, the labels were switched to ensure the blinding and to test the reproducibility as a validation.

### *HPLC conditions for the determination of paclitaxel*

Brand and generic paclitaxel were diluted to 60 μg/mL with pure water, and 20 μL was injected into the high-performance liquid chromatography (HPLC). The HPLC system consisted of an LC-20AT pump, SIL-20AC autosampler, and an SPD-M20A photodiode array detector (Shimadzu, Kyoto, Japan). Sample separation was achieved on a reversed-phase $C_{18}$ column (250 × 4.6 mm ID, 5 μm, Purospher STAR®, Merck, Darmstadt, Germany). The mobile phase was made up of pure water and acetonitrile under a gradient elution mode with a flow rate of 1.0 mL/min. The content of acetonitrile in mobile phase increased linearly from 30% to 90% over 60 minutes and then changed to initial condition to equilibrate for 20 minutes. The system was operated at an ambient temperature and UV wavelength was monitored at 227 nm.

### *Cell viability assessment*

To determine the effect on cell viability, cells were treated with various concentrations of paclitaxel. Cell viability was assessed by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide (MTT) assay.

### *Clonogenic assay*

Viable tumor cells ($10^3$) were plated into each 35 mm culture dish and allowed to grow in McCoy's 5A medium containing 20% heat-inactivated fetal calf serum (FCS) and 0.24% agarose at 37°C in a humidified 5% $CO_2$ incubator. After 10–14 days, the dishes were stained with 3% crystal violet and colonies containing ≥ 50 cells were counted.

### *Cell cycle analysis by flow cytometry*

After 48 hours treatment with paclitaxel, cells were harvested and fixed with 70% ethanol at 4°C for 1 hour. The cells were stained for 30 minutes with propidium iodide solution (propidium iodide, 0.5 mg/mL; RNAse, 0.1 mg/mL) contained in a CycleTEST PLUS DNA reagent kit (Becton Dickinson, Lincoln Park, NJ, USA). Analysis of the DNA content was performed using a FACS Calibur flow cytometer (Becton Dickinson). The data from $10^4$ cells were collected and analyzed using ModFitLT (v.3.0) (Becton Dickinson).

### *Morphological assessment*

Cells were treated with tested drugs, collected, and cytocentrifuged onto a microscope slide using a Cytospin (Shandon Southern Instrument Inc., Sewickly, PA, USA). After stained with Wright's stain, cells were observed under an inverted microscope (Olympus, Hamburg, Germany) at a magnification of 1000×.

### *Statistics*

Data were expressed as mean ± SEM. $IC_{50}$ values for clonogenicity were estimated by GraphPad Prism 4 software (San Diego, CA, USA). The changes in cell viability and cell cycle distribution between Taxol-treated and generic paclitaxel-treated cells were evaluated by the analysis of variance with repeated measurements followed by Bonferroni's test (*$P < 0.05$).

## Results

### *Appearance of drugs*

The appearance of Taxol is transparent and clear, whereas that of generic paclitaxel is light yellow with translucency. While withdrawing the drugs, Taxol seems to have a greater viscosity than that of generic paclitaxel.

### *Analysis of purity by HPLC*

There were no major differences between the amounts of original brand (Taxol) and the generic drugs in solutions inside vials. As shown in Figure 1, the estimated peak area of original brand (Taxol, 60 μg/mL) is 2063877 ± 10683, whereas the peak area of generic brand (60 μg/mL) is 1995057 ± 12642 ($P > 0.05$). The retention time of paclitaxel was 28.5 minutes for both brand and generic forms. Peak identity of Taxol and generic paclitaxel was first recognized by the same retention time. In addition, the UV spectra (from 200 to 400 nm) for Taxol and generic paclitaxel were overlapped with a very similar pattern with a $\lambda_{max}$ at 227 nm. Furthermore, the UV intensity ratio values (254 nm/227 nm) of the peak were compared, and the results showed no significant difference between the original brand form (Taxol) and the generic drug ($P = 0.158$ by $t$-test), suggesting these two peaks were from the same compounds.

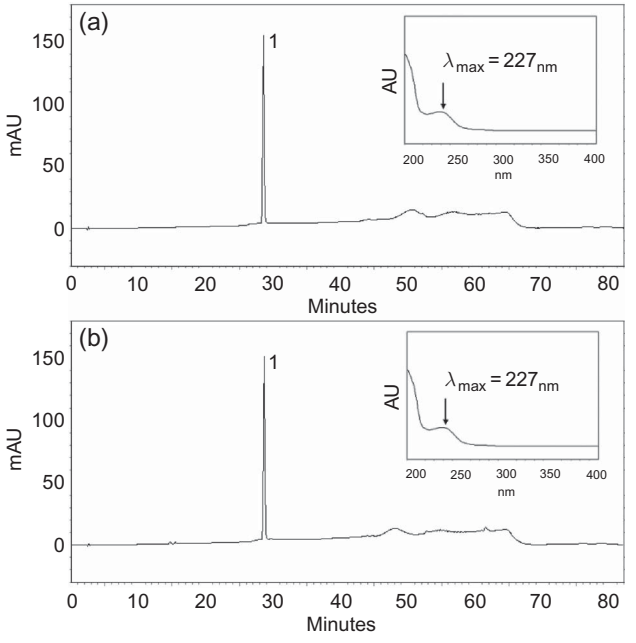


**Figure 1.** Typical chromatograms of paclitaxel. (a) Original brand (Taxol, 60 μg/mL); (b) generic drug (60 μg/mL). The inserted figures indicate the UV spectrum of paclitaxel, which possess maximum absorption wavelength ($\lambda_{max}$) of 227 nm. Peak 1: paclitaxel.

### *Cell viability, clonogenicity, and estimation of $IC_{50}$*

The cell viability assessed by MTT assay showed a greater inhibitory effect on generic paclitaxel than Taxol in both cell lines (Figure 2). To get insight of cell proliferation affected by these two drugs, we further performed clonogenic assay and assessed the dose-dependent effect. For assessment of $IC_{50}$ for clonogenicity inhibition, an index of proliferation, the survival rates of SKOV-3 cells by taxol at 0, 0.008, 0.04, and 0.2 μM are 100%, 86.1%, 64.2%, and 9.5%. Those data for phyxol are 100%, 58.7%, 29.8%, and 3.6 %, respectively. Generic paclitaxel exhibited a lower $IC_{50}$ value (0.025 versus 0.108 μM), indicating a greater inhibitory activity against clonogenicity of human ovarian adenocarcinoma SKOV-3 cells.

### *Cell cycle distribution*

Generic paclitaxel arrested SKOV-3 and ES-2 cells at G2/M phase with concurrent development of hypoploid and polyploid cells, whereas Taxol treatment resulted in a similar pattern but markedly less extent of these changes (Figure 3). As demonstrated in Table 1, the

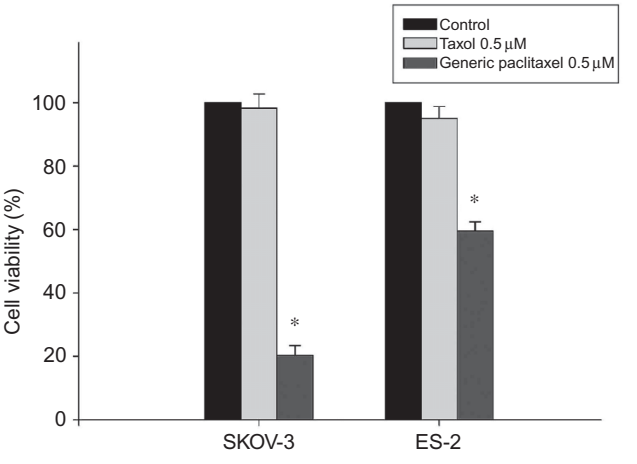


**Figure 2.** Viability of human ovarian cancer cells treated with vehicle, Taxol (0.5 μM) or generic paclitaxel (0.5 μM). After treatment, cells were harvested for MTT assay. Data from three separate experiments are expressed as mean ± SEM. *$P < 0.05$ with comparison with Taxol.

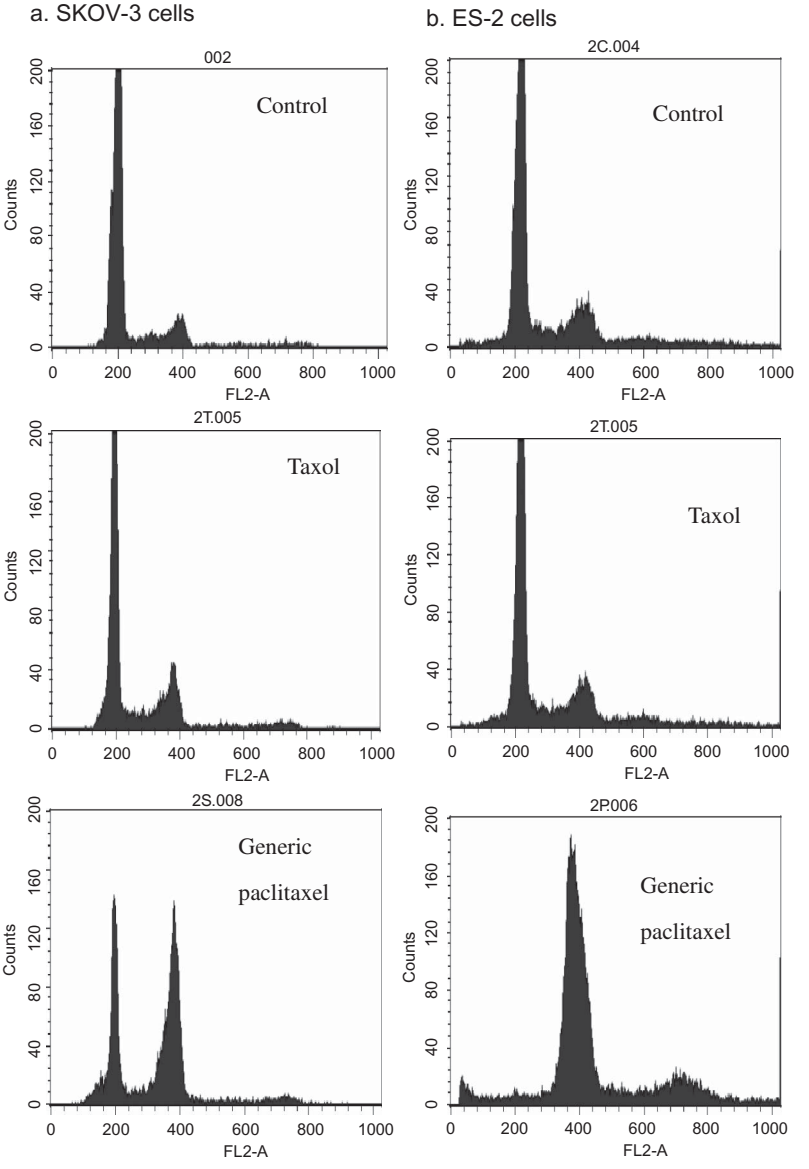


**Figure 3.** Cell cycle distribution of human ovarian cancer cells treated with vehicle, Taxol (0.5 μM) or generic paclitaxel (0.5 μM). (a) SKOV-3 cells; (b) ES-2 cells. Representative flow cytometric analysis for cell cycle distribution 48 hours after the treatment was demonstrated.

**Table 1.** Effect of Taxol versus generic paclitaxel on hypodiploid population and cell cycle distribution in human ovarian cancer cells.

| | | Cell cycle distribution (%) | | |
|---|---|---|---|---|
| | Sub-G1 population (%) | G0/G1 | S | G2/M |
| SKOV-3 | | | | |
| Control | 0.4 ± 0.2 | 83.3 ± 0.7 | 10.0 ± 1.5 | 6.7 ± 0.7 |
| Taxol | 0.8 ± 0.4 | 74.5 ± 3.9 | 16.8 ± 0.8 | 8.7 ± 3.2 |
| Generic paclitaxel | 2.5 ± 0.9* | 27.1 ± 3.6* | 31.2 ± 4.6* | 41.6 ± 4.3* |
| ES-2 | | | | |
| Control | 1.4 ± 0.4 | 74.6 ± 2.9 | 20.2 ± 2.4 | 5.2 ± 0.6 |
| Taxol | 1.6 ± 0.3 | 73.8 ± 2.7 | 18.6 ± 4.5 | 7.6 ± 1.5 |
| Generic paclitaxel | 4.3 ± 1.9* | 1.9 ± 0.4* | 31.6 ± 6.6* | 66.6 ± 6.6* |

After treatment with Taxol or generic paclitaxel (5 μM) for 48 hours, cells were harvested. Cell cycle distribution was analyzed by measuring the DNA content with propidium iodine staining. Data from three separate experiments are expressed as mean ± SEM. *$P < 0.05$ with comparison with Taxol.

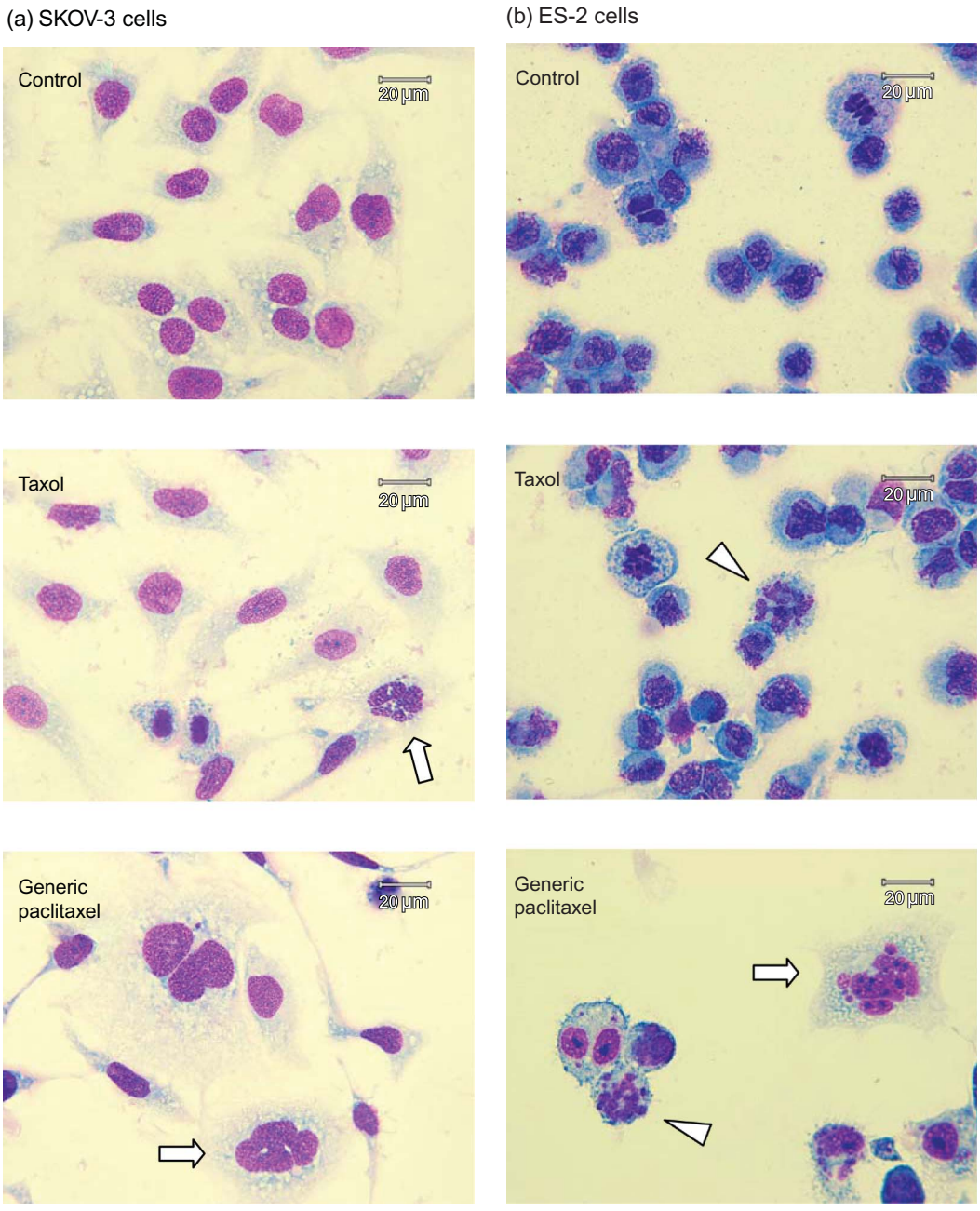


**Figure 4.** Morphology of human ovarian cancer cells treated with vehicle, Taxol (0.5 µM) or generic paclitaxel (0.5 µM). (a) SKOV-3 cells; (b) ES-2 cells. Arrow indicates representative multinucleated cells characteristic of mitotic catastrophe. Arrowhead indicates apoptotic cell (Wright's stain; original magnification 1000×).

percentages of SKOV-3 cells at G2/M phase for control, generic paclitaxel, and Taxol groups were 6.7 ± 0.7, 8.7 ± 3.2, and 41.6 ± 4.3%, respectively. Those for ES-2 cells were 5.2 ± 0.6, 7.6 ± 1.5, and 66.6 ± 6.5%, respectively.

### *Morphological assessment*

As demonstrated in Figure 4, observation of cellular morphology revealed a greater amount of multinucleated (2% versus 1.25% in SKOV-3 cells; 16.5% versus 6.25% in ES-2 cells) and apoptotic (11.8% versus 9.25% in SKOV-3 cells; 47.3% versus 12.3% in ES-2 cells) cells in generic paclitaxel-treated cells, compared to that of Taxol.

## Discussion

To assess the interchangeability of generic and brand name drugs of paclitaxel in a hospital-based laboratory,

we tried to examine their purity and in vitro antitumor activity against human ovarian cancer cells. We found that generic paclitaxel, having the same purity as Taxol, possessed a greater viability and clonogenicity inhibitory activity against human ovarian cancer cells than the original brand Taxol. Generic paclitaxel induced apoptosis, arrested cells at G2/M phase, and resulted in multinucleated cells in both cell lines with an extent greater than Taxol.

The clonogenicity assay has been used to assess the reproductive integrity of tumor cells[6,7]. MTT assay measures the amount of formazan coming from the oxidization activity in viable cells possessing functional mitochondria[8,9]. Generic paclitaxel exhibits a greater inhibitory effect on viability and clonogenicity of ovarian cancer cells. Taken together, it suggests that generic paclitaxel may inhibit the reproductive integrity, an in vitro model for tumorigenesis in vivo, of human ovarian cancer cells.

The mode of cell death induced by generic paclitaxel and Taxol seems different. Besides apoptosis, generic paclitaxel induces a population of multinucleated cells, resembling cells undergoing mitotic catastrophe. The development of this mode of cell death is further confirmed by the accumulation of polyploidy cells assessed by DNA histogram. Taxol induces a less prominent population of mitotic catastrophe-like cells and a scanty amount of apoptotic cells.

After expiration of patent protection of original brand, generic products usually are marketing at a lower price after drug approval. Other than the consideration of cost-effectiveness, the therapeutic effectiveness remains the major concern for interchangeability between brand and generic or between various sources of generic drugs. To evaluate the therapeutic effectiveness, conduction of clinical trials is the mainstay way to address this issue[10]. However, it is not practical to conduct clinical studies for each interchange in a hospital. Results of this study may provide a clue for assessing the interchangeability by the examination of both in vitro purity and bioactivity in a hospital-based laboratory.

Our results indicate that generic drug of paclitaxel might possess greater growth and clonogenicity inhibitory activity against its corresponding brand for ovarian cancer cells. This may support the interchangeability of these drugs in our hospital, not only by consideration of cost. However, one should be very cautious while interpreting the meaning of these data. Before making decision according to these data, investigators should ensure that these experiments are performed under a blinded manner with reproducible results by label switch in some experiments.

## Conclusion

By using paclitaxel as an example, we conclude that this experimental model for the assessment of the interchangeability of generic and brand name drugs might be considered as a clue and could be easily established in a hospital-based laboratory. The cause for different activities of these two drugs with identical HPLC profiles is still unclear. The differences in composition or amount of excipients could be part of the potential solutions. Further studies for head-to-head comparison between these two drugs remain crucial to validate these differences.

## Declaration of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this paper.

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